















SIXTH REPORT ON PLAGUE  
INVESTIGATIONS IN INDIA

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C. F. CLAY, MANAGER



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THE  
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PLAGUE SUPPLEMENT I

SIXTH REPORT ON PLAGUE  
INVESTIGATIONS IN INDIA

ISSUED BY

THE ADVISORY COMMITTEE

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## CONTENTS

(Continued from Volume X, p. 568.)

	PAGE
XL. Major George Lamb, M.D. Glasg., I.M.S. With Portrait (Plate I) . . . . .	2
XLI. Epitome of some recent observations on rat fleas . . .	7
XLII. Preliminary observations on the protective and curative value for rats of the serum of a horse immunised with a toxic nucleo-protein extracted from the plague bacillus. By Sydney Rowland . . . . .	11
XLIII. Second report on investigations into plague vaccines. By Sydney Rowland. (With 1 Diagram) . . .	20
XLIV. Statistical investigation of plague in the Punjab. Second report: On the connection between proximity to rail- ways and frequency of epidemics. By M. Greenwood, Junr. (With Map) . . . . .	47
XLV. Statistical investigation of plague in the Punjab. Third report: On some of the factors which influence the prevalence of plague. By M. Greenwood, Junr. (With Map and 5 Figures) . . . . .	62
XLVI. Observations on plague in Eastern Bengal and Assam. (With Map and 5 Charts in Text and Plates II-XIV)	157
XLVII. Observations on the breeding of <i>Mus rattus</i> in captivity. (With 1 Chart) . . . . .	193



VII

SEVENTH REPORT ON PLAGUE  
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*(Continued from Volume XI, Plague Supplement.)*

## CONTENTS

	PAGE
XLVIII. Plague in Madras City. (With 1 Chart) . . .	207
XLIX. Statistics of the occurrence of plague in man and rats in Bombay, 1907-11. . . . .	221
L. The distribution of white-bellied <i>Mus rattus</i> in Bombay Island. (With 1 Map) . . . . .	227
LI. The immunity of the wild rat in India. (With 1 Map) . . . . .	229
LII. Chronic or resolving plague. (With 1 Chart and Plate XV) . . . . .	266
LIII. The experimental production of resolving plague in rats . . . . .	287
LIV. Experimental plague epidemics among rats . . .	292
LV. Observations on flea-breeding in Poona. (With 7 Charts) . . . . .	300
LVI. The serum treatment of human plague . . .	326
LVII. Attempt to separate the antigen from the nucleo- protein of the plague bacillus by filtration through gelatin. By Dr S. Rowland . . .	340

## *Contents*

	PAGE
LVIII. Besredka's method of vaccination. By Dr S. Rowland	344
LIX. The relation of pseudotubercle to plague as evidenced by vaccination experiments. By Dr S. Rowland	350
LX. Observations on the mechanism of plague immunity. By Dr S. Rowland . . . . .	358
LXI. The onset and duration of the immunity consequent on the inoculation of plague nucleoprotein. By Dr S. Rowland. (With 2 Charts) . . . . .	367
LXII. The opsonic index in plague vaccination. By Dr R. St John Brooks. (With 5 Charts) . . . . .	373
LXIII. The preparation of antitoxic plague sera. By Dr A. T. MacConkey . . . . .	387



XLV

EIGHTH REPORT ON PLAGUE  
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*(Continued from Volume XII, Plague Supplement.)*

## CONTENTS

	PAGE
LXIV. The influence of cultivation in serum-containing media upon the virulence and immunising properties of the plague bacillus. By S. Rowland. (With Plate XVI) . . . . .	403
LXV. The influence of the medium in which the plague bacillus is propagated upon the facility with which it is ingested by human leucocytes. By R. St John Brooks . . . . .	412
LXVI. The morphology of the plague bacillus. By S. Rowland. (With Plates XVII—XXIII) . . . . .	418
LXVII. Observations on the mechanism of the transmission of plague by fleas. By A. W. Bacot and C. J. Martin. (With Plates XXIV—XXVI and 4 Text-figures) . . . . .	423
LXVIII. Influence of the medium in which <i>B. pestis</i> is propagated upon its virulence. By S. Rowland . . . . .	440

# Contents

	PAGE
LXIX. A study of the bionomics of the common rat fleas and other species associated with human habitations, with special reference to the influence of temperature and humidity at various periods of the life history of the insect. By A. W. Bacot. (With Plates XXVII—XXXIV, 12 Charts and 3 Text-figures) . . . . .	447
LXX. On the survival of bacteria in the alimentary canal of fleas during metamorphosis from larva to adult. By A. W. Bacot . . . . .	655
LXXI. The effect of the vapours of various insecticides upon fleas ( <i>Ceratophyllus fasciatus</i> and <i>Xenopsylla cheopis</i> ) at each stage in their life history and upon the bed bug ( <i>Cimex lectularius</i> ) in its larval stage. By A. W. Bacot. (With 1 Text-figure) . . . . .	665

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*(Continued from Volume XIII, Plague Supplement.)*

### CONTENTS

	PAGE
LXXII. Epidemiological observations in Madras Presidency. By Captain J. C. Kunhardt, I.M.S., and Captain J. Taylor, I.M.S., assisted by Assistant-Surgeons R. Ganpati Iyer, T. Kesava Menon, B. V. Varadhachari, R. Raghavendra Rao and K. Narayan Rao. (With 7 Maps and 30 Charts)	683
I. Administrative Divisions . . . . .	685
II. Physical features . . . . .	687
III. Climatic conditions . . . . .	688
IV. Distribution of plague . . . . .	708
V. Influence of climate on distribution of plague .	713
VI. Rat and flea prevalence . . . . .	725
VII. Experiments to test the susceptibility of rats to plague infection . . . . .	744
VIII. Measures adopted for the prevention of plague .	745
LXXIII. Further experiments on vaccination against a body-strain of plague. By S. Rowland . . . . .	752
LXXIV. Immunisation by pseudotubercle. By S. Rowland . . . . .	754

	PAGE
LXXV. Immunisation by living avirulent cultures (Strong). By S. Rowland . . . . .	756
LXXVI. The influence of race on the efficiency of the antigen. By S. Rowland . . . . .	759
LXXVII. On the failure to vaccinate against a virulent body-strain even with an antigen prepared as far as possible under body conditions. By S. Rowland . . . . .	760
LXXVIII. The protective and curative value, against infection with a serum race of plague, of the serum of a horse immunised with nucleoprotein extracted from a strain of plague bacilli propagated on serum protein. By S. Rowland . . . . .	762
LXXIX. Ultraviolet light as a germicide in the preparation of plague vaccine. By S. Rowland . . . . .	765
LXXX. Observations on the length of time that fleas ( <i>Ceratophyllus fasciatus</i> ) carrying <i>Bacillus pestis</i> in their alimentary canals are able to survive in the absence of a host and retain the power to re-infect with plague. By A. W. Bacot . . . . .	770
LXXXI. Further notes on the mechanism of the transmission of plague by fleas. By A. W. Bacot. (With Plates XXXV and XXXVI and 2 Text-figures)	774
LXXXII. Notes on the development of <i>Bacillus pestis</i> in bugs ( <i>Cimex lectularius</i> ) and their power to convey infection. By A. W. Bacot. (With Plates XXXVII and XXXVIII and 1 Text-figure) . . . . .	777

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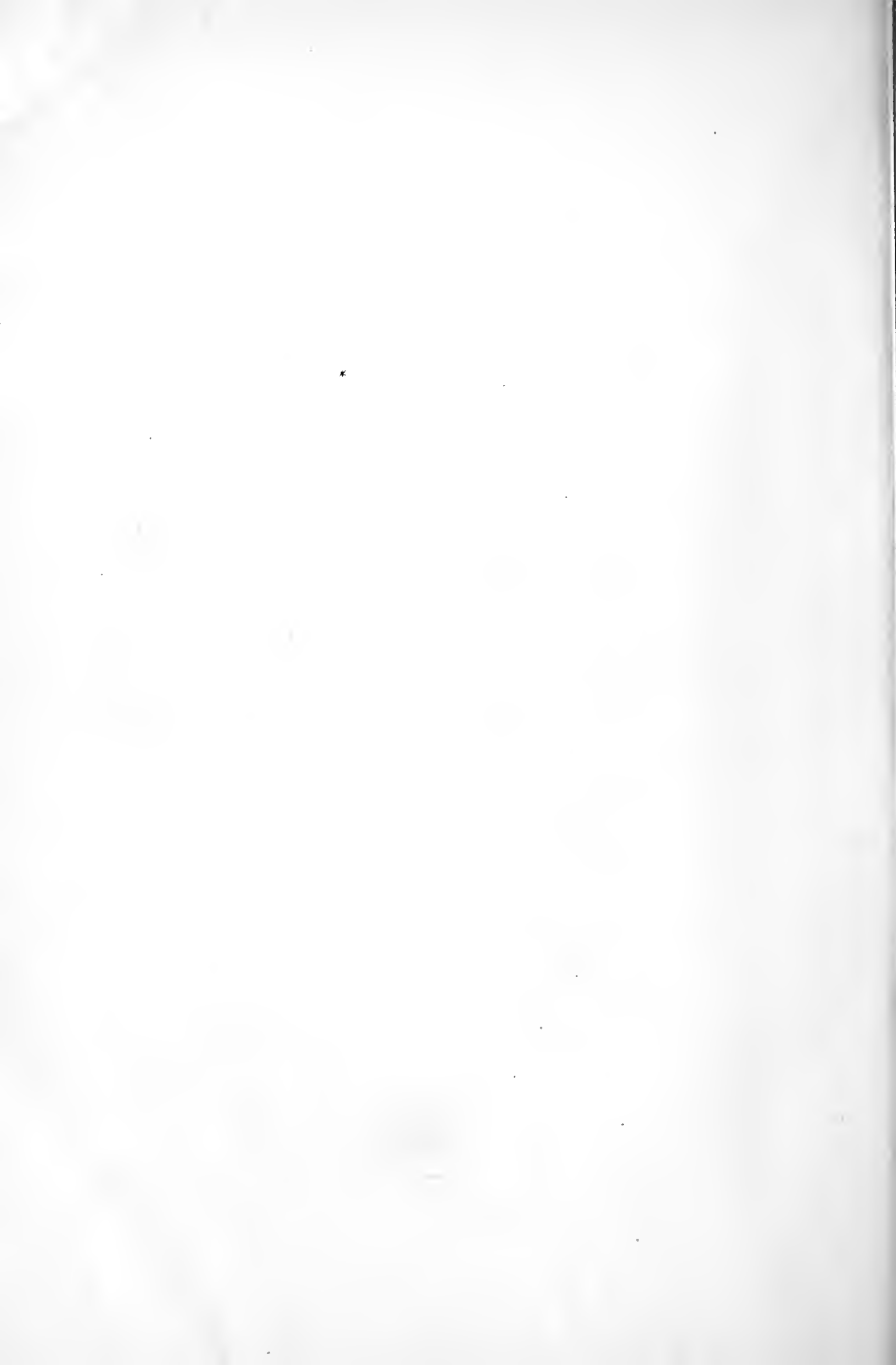
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ROYAL SOCIETY, AND THE LISTER INSTITUTE.

*(Continued from Volume XIV, Plague Supplement.)*

CONTENTS

	PAGE
LXXXIII. Epidemiological observations in the United Provinces of Agra and Oudh, 1911—1912. By Major T. H. Gloster, I.M.S., and Major F. N. White, I.M.S., assisted by Assistant-Surgeons A. N. Mukharji, J. S. Roy Chaudhuri and C. C. Mitra, and Sub-Assistant-Surgeons G. C. Mandal and Manji Ram. (With 4 Maps and 9 Charts)	793
I. Introduction	793
II. Observations in Lucknow	817
III. Observations in Cawnpore	824
IV. Observations in the Ballia District	832
V. Observations in the Bundelkhand	850
VI. The Severe Epidemics of Plague in Muttra in 1904-5 and in Muzaffarnagar in 1906-7	868
LXXXIV. The influence of saturation deficiency and of temperature on the course of epidemic plague. By Ralph St John Brooks. (With 18 Charts)	881



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XL. Major George Lamb, M.D. Glasg., I.M.S. . . . .	2
XLI. Epitome of some recent observations on rat fleas . . . . .	7
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XLIII. Second report on investigations into plague vaccines. By Sydney Rowland . . . . .	20
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XLVI. Observations on plague in Eastern Bengal and Assam.	157
XLVII. Observations on the breeding of <i>Mus rattus</i> in cap- tivity . . . . .	193
Journ. of Hyg.	1

## XL. MAJOR GEORGE LAMB, M.D. GLASG., I.M.S.<sup>1</sup>

With Portrait (Plate I).

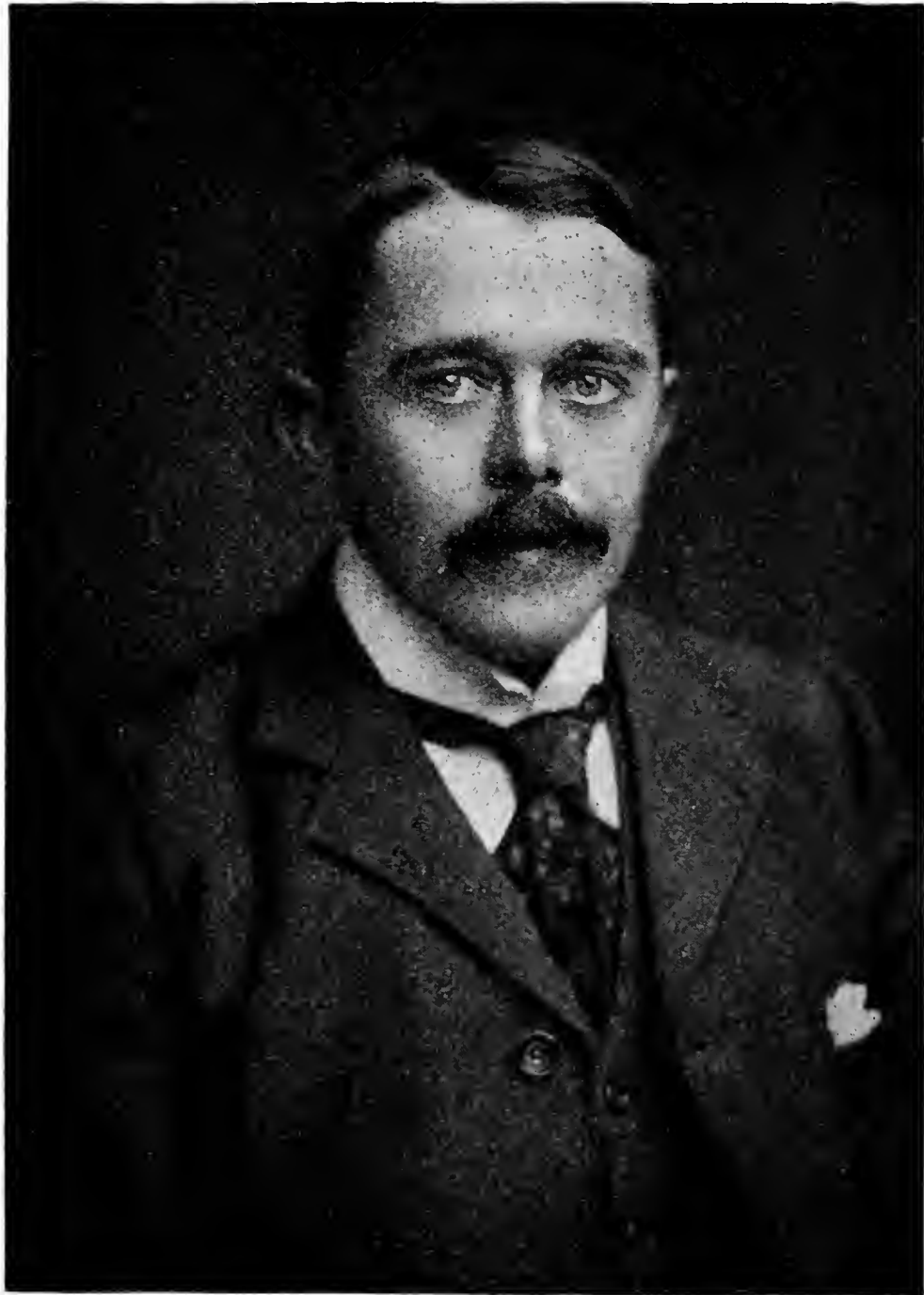
By the death of Major Lamb the Indian Medical Service has lost one of its ablest and most esteemed members, and science has been robbed of one of her most ardent devotees.

Dr Lamb was a man of great scientific attainments and, although but 41 years of age, had already achieved a reputation which is by no means confined to India. His contributions to science are as well known and as highly appreciated by scientific men throughout the world as by his colleagues in the Service. His early death, at a period when his judgment had matured but his imagination and enthusiasm remained unimpaired, is a serious loss to the small band of earnest scientific investigators in India, and one which will not easily be made good.

Lamb was educated at the Glasgow High School, whence he proceeded to the University of Glasgow in 1886. He had a brilliant university career, and on graduating in 1890 received the Bruton Memorial Prize as the most distinguished graduate of his year. On taking the degree of M.D. a few years later, he was awarded the Bellahouston Gold Medal. After a year spent in filling resident appointments at the Western Infirmary under Sir Hector Cameron and Sir William Gairdner, he became Demonstrator of Anatomy under Prof. Cleland. This position he relinquished in 1893 when he entered the Indian Medical Service, obtaining the first place in the competitive entrance examination. At Netley he obtained most of the prizes available, and at the conclusion of the course again took the first place.

At Netley, Lamb met Sir Almroth Wright, who was then Professor of Pathology at the Army Medical School. The direction of his interests towards pathology was due, in large measure, to Prof. Wright's influence. Lamb owed much to the inspiration gained from this teacher, and was always pleased to express his indebtedness and to insist with charming enthusiasm on the great stimulus Wright's teaching had been to him.

<sup>1</sup> The substance of this notice first appeared in the *British Medical Journal*, 29 April, 1911.



George Lamb

---



In April 1894 Lamb proceeded to India, and for the next three years was employed in military service, mostly on the North-West Frontier, where he took part in the Waziri expedition. He could hardly have conformed to all the conventional ideas of a military surgeon, but he was very popular with his brother officers, and greatly esteemed by the troops under his medical care. These consisted for the most part of men recruited from the hill tribes—wild creatures whose morals and ideals differed no doubt from average European standards, but for whom, nevertheless, he entertained a considerable measure of respect. On the Frontier, especially under active service conditions, Lamb encountered life stripped of most of its affectations, an experience which is always educative and salutary, and especially so to one who has just spent seven years of academic existence. Although often at the time chafing to be again at scientific work, he was wont to look back upon his three years spent as a military surgeon on the Frontier without regret and with evident enjoyment.

In 1897 he returned to Europe on six months' leave, which he spent in study at the Pasteur Institute in Paris. On the termination of his leave, he was delighted to find that he had been selected by the Government of India to proceed to the Royal Army Medical School at Netley for further training in pathology and bacteriology, and to assist his former teacher, Prof. Wright, in the teaching work of the department of pathology. This happy arrangement lasted for eighteen months, and Lamb always regarded it as the most joyful time of his life. The admiration he had conceived for Wright, when a student at the Army Medical School, rapidly developed into the warmest affection, with the closer intimacy which he now enjoyed with his chief. The teaching duties were neither arduous nor continuous, and the major portion of his time and energy was available for research. The conditions in the laboratory were most congenial. The other assistants were Major Semple (now Sir David Semple) and Captain Birt (now Colonel Birt), with both of whom he collaborated in researches and formed close friendships, which lasted throughout life. Whilst at Netley, Lamb published three valuable papers dealing with various aspects of immunity, which showed that the brilliant student had become a capable investigator.

Returning to India in 1899, he became assistant to Prof. Haffkine, at that time Director of the Bombay Bacteriological Laboratory at Parel. In addition to numerous duties connected with his post, Lamb now commenced a series of investigations into the action of Indian

snake venoms, for which he is justly renowned. These researches, which at first dealt with the nature and physiological action of the poisons produced by the different serpents of India, were later concerned with the production, properties and standardization of anti-venomous serums, and brought to light many facts of fundamental importance to the doctrine of immunity. The results appeared in twenty papers published between 1899 and 1907, partly in the *Scientific Memoirs* of the Government of India and partly in the *Lancet*. These researches, which involved the analysis of the action of the different poisons upon the various mechanisms of the animal body, necessitated the employment of very various physiological and pathological techniques. They were carried out in laboratories in India which were by no means sumptuously equipped for such inquiries. Nevertheless, they are models of insight and accuracy, and form a monument which will endure in the annals of scientific achievement. At the commencement of these inquiries Lamb was himself bitten by a cobra whilst manipulating the reptile in the laboratory, and narrowly escaped with his life.

It was about this time that I first met Lamb. His earlier researches on snake poison had led to a correspondence between us, and in 1901 it was arranged that we should meet in Bombay. I recall with vivid distinctness our first meeting. It was in one of the quaint laboratories transformed out of old Government House, Parel, which had previously been a Portuguese Jesuit seminary. It smelt strongly of teak, of which the fittings were constructed. I was enchanted by the simple frankness and geniality which contributed so greatly to the charm of his personality, and within a few minutes we seemed to be old friends. It was, however, his extraordinary keenness which impressed me most. Making every allowance for the intensification of the impression by the background—the climate of Bombay does not conduce to the manifestation of acute mental alertness and enthusiasm—Lamb struck me as the keenest man I had ever met. I remember we spent most of the night in a half-naked condition discussing snake poisons, and that it was one of the most entertaining conversations I have ever enjoyed.

Dr Lamb left Bombay in 1903, and spent the next few years at Kasauli, where he was assistant to Colonel Semple at the Pasteur Institute of India. Shortly before leaving Parel he married Patricia Liston, the sister of his old friend and colleague, Major Glen Liston.

The effect of strenuous work in Bombay had been very trying, and he left that picturesque city regretting only its glorious harbour, for he was an enthusiastic sailor-man, and his only recreation was sailing his



*Tom-Tit.* Kasauli, being situated on the summit of one of the foot-hills of the Himalayas, enjoys a delightful climate, excellent for children, and addiction to laboratory work may be indulged with as little injury to health as in this country. The isolation, too, was congenial both to Mrs Lamb and himself, and he looked forward to much quiet happiness and increasing opportunities for scientific work. Although at Kasauli the work of the Pasteur Institute, where about 1000 patients were annually treated for rabies, made greater demands upon his time than had the duties of the post at Parel, this was more than compensated for by the improved climatic conditions. The snake-poison work was continued and experiments on the standardization of antityphoid vaccines and other researches incidental to the work of the institute were also carried out.

Before leaving Bombay, Lamb had discovered the existence of Mediterranean or Malta fever in India, and was inclined to believe that many of the obscure cases of fever amongst native troops, hitherto indiscriminately ascribed to malaria, were really cases of this disease. This view was warmly combated in many quarters. At Kasauli, however, he had the opportunity of studying some suspicious outbreaks amongst troops in the Punjab, and succeeded not only in justifying his contention, but showed that the disease was brought about by the consumption of infected goat's milk, as had recently been ascertained to be the case in Malta by the Mediterranean Fever Commission.

In 1905, Colonel Semple having been transferred to the new Central Institute for Research recently built at Kasauli, Dr Lamb succeeded to the position of Director of the Pasteur Institute. At the same time he gained his majority by accelerated promotion on the ground of his distinguished services.

He had not long held this responsible position, however, when the Advisory Committee asked the Government of India to place the services of Major Lamb at their disposal. The Government of India could not at first see its way to comply with this request. The committee was convinced, however, that Lamb was essentially the man to be the senior member of the Commission, and after a good deal of importunity their request was acceded to, with results which more than justified their expectations.

The Council of the British Medical Association, recognizing the importance of Major Lamb's share in the results of the work of the Commission, last summer awarded him the Stewart Prize for the most

important contribution made within the last few years to our knowledge of the spread of a disease.

In this appreciation of Major Lamb the scientific side of his career has been more particularly represented. His death will also be a serious grief to a large number of friends and acquaintances, who came to love and admire him as a man, and among those who will mourn his loss most acutely will be his old bearer, who had faithfully served him since he came to India, and whom I last saw tending with the same pride and care the little sahibs of the next generation.

C. J. M.

## XLI. SOME RECENT OBSERVATIONS ON RAT FLEAS.

### I. *The fleas common on rats in different parts of the world.*

CHICK and Martin<sup>1</sup> have recently published a valuable summary of our knowledge on this subject and give full references to the literature. The chief data may be shortly summarised as follows:—

Locality	Fleas examined	Percentage of total fleas belonging to each species					
		<i>Xenopsylla cheopis</i>	<i>Cerato-phyllus fasciatus</i>	<i>Cerato-phyllus anisus</i>	<i>Cteno-psylla musculi</i>	<i>Cteno-phthalmus agyrtes</i>	Other species
India	891,000	100	trace	—	—	—	—
Australia	2,000	63	11	—	24	—	2
San Francisco	31,000	28	58	—	8	—	6
Japan	8,000	18	—	46	24	—	12
Mediterranean	13,500	58	25	—	11	—	6
England	1,000	—	48	—	—	52	—

The proportionate figures represent only the relative prevalence of the different kinds in the exact circumstances under which the sample of rats and fleas was obtained. Some differences in locality may materially alter the flea population; thus at Marseilles and in Japan it has been clearly shown that *X. cheopis* is much more frequent in connection with shipping than in more truly indigenous foci. The general prevalence of *X. cheopis* about the world is therefore probably exaggerated by the figures given, which apart from India and England mostly refer to seaports.

Other fleas, which are not primarily rat fleas, also occur on rats: cat (*Ct. felis*), dog (*Ct. canis*), and human (*P. irritans*) fleas have been found, usually in small numbers, by many observers and several other species have been recorded. In Italy as many as 25% or 30% of the fleas found on rats have been observed to be cat and dog fleas, and in German East Africa a fifth was the fowl flea (*Echidnophaga gallinacea*). In

<sup>1</sup> *Journal of Hygiene*, Vol. xi. (1911), p. 122.

this locality *Xenopsylla scopulifer* (*X. brasiliensis*) also occurred pretty freely<sup>1</sup>. No evidence has been obtained that, other conditions being the same, the flea population on *Mus rattus* is different from that on *M. decumanus*.

## II. The occurrence of *Xenopsylla cheopis* in England.

Chick, Martin and Rowland have found that the fleas on wild country rats in Suffolk, Hertfordshire and Devonshire are about equally divided between *C. fasciatus* and *Ct. agyrtes*. In Suffolk, Martin and Rowland got 584 fleas off 568 rats, the highest prevalence in any one locality being  $3\frac{1}{2}$  fleas per rat<sup>2</sup>. A single specimen of *X. cheopis* was taken at Plymouth some years since<sup>3</sup>, but otherwise the species has not been found in this country. The following data therefore regarding the fleas found on *M. decumanus* trapped in Guy's Hospital, London, are of some interest<sup>4</sup>. Locality *B* is distant rather less than 100 yards from locality *A*.

### Locality *A*.

Date	Rats caught	<i>C. fasciatus</i>	<i>X. cheopis</i>
1. 2. 11	1 big	0	0
3. 2. 11	6 young	18	1
6. 2. 11	1 „	9	1
14. 2. 11	1 big	2	0

### Locality *B*.

10. 3. 11	1 big	0	0
17. 3. 11	1 young	0	30
21. 3. 11	2 „	0	75
22. 3. 11	1 „	3	49
22. 3. 11	1 „	1	3
23. 3. 11	2 „	0	73
12. 4. 11	1 big	0	9
13. 4. 11	2 „	1	66
26. 4. 11	1 young	1	36
30. 8. 11	1 „	1	41
31. 8. 11	1 „	3	18
31. 8. 11	1 big	2	14

<sup>1</sup> By an error in tabulation Chick and Martin enter this flea as having been recorded by the Commission from Poona (Vol. x. p. 524); the record refers to *P. irritans*.

<sup>2</sup> *Reports to the Local Government Board on Public Health and Medical Subjects*, New Series, No. 52 (1911), p. 49.

<sup>3</sup> Giles, *Entomol. Monthly Mag.* Vol. xli. (1905), p. 139.

<sup>4</sup> *Guy's Hospital Gazette*, Vol. xxv. (1911), pp. 73, 318; *Entomol. Monthly Mag.* (2nd series), Vol. xxii. (1911), pp. 68, 113.

Thus in locality *A*, 94 % were *C. fasciatus*, while in locality *B*, 97 % were *X. cheopis*. In all 24 rats yielded 457 fleas, an average of 19 fleas per rat.

### III. The biting of man by rat fleas.

Chick and Martin<sup>1</sup> have made a number of interesting experiments on the readiness with which the various species of rat flea bite human beings and animals. Each flea was given an opportunity of biting for two minutes. The results may be summarised as follows:—

	Number of experiments	Percentage of positive results
<i>C. fasciatus</i> : man	517	60
„ rat	101	58
„ rabbit	32	72
<i>Ct. musculi</i> : man	111	4
„ mouse	11	82
<i>Ct. agyrtes</i> : man	98	none
„ rat	19	58

*C. fasciatus* therefore bites man quite readily, while *Ct. musculi* and *Ct. agyrtes* do so hardly or not at all. A number of observations have shown that the race of *X. cheopis* occurring in London bites men greedily, even immediately after being taken off a rat (see Vol. VII. p. 472, VIII. p. 239). Thus off a freshly-caught rat seven *C. fasciatus* and 13 *X. cheopis* were obtained with chloroform at 9.45 a.m. At 12.30 they had recovered from the anaesthetic and were given the opportunity of biting the human arm: within five minutes all the *C. fasciatus* and 11 of the *X. cheopis* bit.

### IV. The seasonal prevalence of rat fleas.

Gauthier and Raybaud<sup>2</sup> have published numerical data with respect to the various sorts of fleas taken off rats at Marseilles at different times of the year. Their figures for *X. cheopis* are as follows, together with the average mean monthly temperature and humidity:—

Month	<i>X. cheopis</i>	Temperature	Humidity
January	39	44° F.	68 %
February	6	47	64
March	5	50	61
April	12	55	60
May	2	61	59
June	2	68	57
July	21	72	54
August	32	71	57
September	50	67	63
October	39	59	69
November	26	51	71
December	24	45	70

<sup>1</sup> *Journal of Hygiene*, Vol. xi. (1911), p. 129.

<sup>2</sup> *C. R. Soc. Biol.* Vol. LXVIII. (1910), pp. 196, 198.

The numbers on which the flea figures are based are not very large and the results are irregular; they indicate however sufficiently clearly a great increase of *X. cheopis* during the autumn. Plague used to prevail in the south of France at that season. For the other species of fleas their figures are still smaller, and the results very irregular. On the whole however they suggest that the abundance of *C. fasciatus* and *Ct. musculi* varies in much the same way.

Tidswell<sup>1</sup> has published similar figures for Sydney, based on the examination of 26,744 rats of which 177 were plague infected and off which 1926 fleas were obtained (62% being *X. cheopis*).

Month	Fleas per 1000 rats			Average mean monthly temperature	Average mean monthly humidity
	<i>X. cheopis</i>	<i>C. fasciatus</i>	<i>Ct. musculi</i>		
January	57	26	43	72° F.	70 %
February	50	4	34	71	73
March	112	11	26	69	75
April	97	2	13	65	78
May	31	1	11	58	77
June	28	2	13	54	79
July	21	5	10	52	77
August	5	0	10	55	74
September	50	3	40	59	70
October	8	20	27	63	68
November	2	3	8	67	68
December	3	10	11	70	68

The plague season here is January to July with a maximum in March, April and May, so that there is good correspondence with the season of maximum prevalence of *X. cheopis*. Though all three species of flea seem to take part in the small autumnal increase, the great spring prevalence affects *X. cheopis* more than the others. This might, if confirmed, constitute an important epidemiological consideration and would reduce the significance of the fact that *C. fasciatus* bites men so readily.

In Japan<sup>2</sup>, Kitasato has found that the absolute and relative abundance of *X. cheopis* is much increased during the autumn, *i.e.* during the plague season.

<sup>1</sup> Report of the Government Bureau of Microbiology for 1909 (Sydney, 1910), p. 20.

<sup>2</sup> Trans. Bombay Med. Congress, 1909, p. 93.

XLII. PRELIMINARY OBSERVATIONS ON THE PROTECTIVE AND CURATIVE VALUE FOR RATS OF THE SERUM OF A HORSE IMMUNISED WITH A TOXIC NUCLEO-PROTEIN EXTRACTED FROM THE PLAGUE BACILLUS.

By SYDNEY ROWLAND, M.A., M.R.C.S.

*Of the Lister Institute.*

IN a previous report (Vol. x. p. 536) I have dealt with a method of extracting from the plague bacillus a substance or substances which appear to be responsible both for the acute toxic action and immunising properties of killed plague bacilli. I have since investigated the properties of the serum of a horse immunised with this solution. In this inquiry I have had the advantage of the assistance of Dr A. T. MacConkey, Head of the Serum Department of the Lister Institute, who kindly undertook the supervision of the immunisation of the horse and to whom I desire to express my thanks.

For the purposes of the experiment a horse was selected and immunisation commenced on June 26, 1909. The material injected into the horse consisted of nucleo-protein dissolved in dilute saline prepared as explained in my previous report, where it was designated solution *B*. The method of immunisation adopted was as far as possible that usually employed in the Serum Department of the Lister Institute for the preparation of diphtheria antitoxin. The table gives the dates and doses administered intramuscularly. Unfortunately owing to lack of toxin the intervals towards the end of immunisation were longer than were absolutely necessary.

The local reaction after inoculation was almost identical with that observed after inoculation of diphtheria toxin. Some tenderness was present and swelling and oedema varying in amount, with transitory

constitutional disturbance and little temperature reaction. There was no tendency to abscess formation or to the huge hard swellings of long duration which are not infrequently the result of injection of unfiltered cultures.

TABLE.

Date	Dose in mgs.	Date	Dose in mgs.
June 26	0·01	July 26	28·0
28	0·02	Aug. 3	42·0
July 3	0·1	10	80·0
7	0·2	24	100·0 Sample bleeding
9	0·4	Sept. 9	15·0 taken.
13	0·8	Oct. 26	30·0
15	1·6	Nov. 13	60·0
17	1·6	20	120·0
20	3·5	Dec. 8	240·0
22	7·0	29	Bled 8 litres.
24	14·0		

*Neutralisation of the toxic extract (solution B) by means of the serum.*

The first point to settle was whether the serum contained any substance capable of neutralising the toxin contained in solution B, i.e., the toxin with which the horse had been immunised.

*Method.* The method used to investigate this point was to mix a known number of lethal doses of the toxin (M.L.D. = 0·1 mg. nucleo-protein) with a known volume of the serum, to leave the mixture at 35° C. for 30 minutes and then to inject it subcutaneously into rats and observe the result. If the rat lived, it was considered that the toxin had been neutralised.

*Experiment 1<sup>1</sup>.*

Rat	No. of L.D. of toxin	Serum in c.c.	Result
1 }	10	0·1	—
2 }			—
3 }	25	0·1	+ 1 day
4 }			+ 1 day

1/10th c.c. of serum neutralises 10 lethal doses of toxin but not 25.

<sup>1</sup> Throughout these records — or 0 = lived, + = died.



*Experiment 2.*

Rat	No. of L.D. of toxin	Serum in c.c.	Result
1)	5·0	0·1	—
2)			—
3)			—
4)	7·5	0·1	—
5)			—
6)			—
7)	10·0	0·1	—
8)			+ 1 day
9)			+ 1 day
10)	12·5	0·1	+ 1 day
11)			+ 2 days
12)			+ 2 days
13)	15·0	0·1	+ 1 day
14)			+ 1 day
15)			+ 1 day
16)	17·5	0·1	+ 1 day
17)			+ 1 day
18)			+ 1 day

The serum used in this experiment was from a second bleeding 5 days after the first bleeding. 1/10th c.c. of this serum neutralised 7·5 lethal doses of toxin.

In these experiments (1 and 2) the dose of serum was kept constant and the dose of toxin varied; in the next series the dose of toxin was kept constant and the dose of serum varied.

*Experiment 3. Estimation of the amount of serum required to neutralise 10 lethal doses of toxin B.*

Rat	Serum in c.c.	Result
1)	0·08	+ 1 day
2)		+ 1 day
3)		+ 1 day
4)		—
5)		—
6)	0·1	+ 1 day
7)		+ 1 day
8)		+ 1 day
9)		—
10)		—
11)	0·12	—
12)		—
13)		—
14)		—
15)		—
16)	0·14	—
17)		—
18)		—
19)		—
20)		—

10 lethal doses toxin were neutralised (*qua rat*) every time by 0·12 c.c. serum and four out of ten times by 0·1 and 0·08 c.c. serum.

It having been shown that the amount of serum necessary to neutralise 10 lethal doses of the toxin, when mixed with it before injection into a rat, lies between 0·1 and 0·12 c.c., we have now to determine whether this ratio still holds when dealing with a greater number of lethal doses of the toxin. For this purpose 100 lethal doses of toxin were mixed with the quantities of serum shown in the following table: the mixture stood for one hour at 37° C. and was then injected into rats.

*Experiment 4.*

Rat	No. of L.D. of toxin	Serum in c.c.	Result
1 } 2 } 3 } 4 } 5 } 6 } 7 } 8 }	100	2 1·75 1·5 1·25	Survived ,, ,, ,,

From this experiment it is evident that the value of the ratio is maintained.

We conclude then *that the serum of this horse can neutralise the toxin in solution B.*

As affording some information on the progress of immunisation we give here the result of a test made, about half-way through the immunisation process, of a sample bleeding on August 24th.

*Experiment 5.*

Rat	L.D. toxin	Serum in c.c.	Result
1 } 2 }	102	1·0	+ 1 day + 1 day
3 } 4 }	68	1·0	+ 2 days + 3 days
5 } 6 }	34	1·0	- -
7 } 8 }	3·4	1·0	- -

At this time 1 c.c. of serum neutralised 34 lethal doses of toxin, compared with between 85 and 100 at the time of the final bleeding.

*Effect of Yersin serum on the toxin in solution B.*

It is necessary to compare with this serum that obtained by Yersin's method of immunisation, *i.e.* the ordinary anti-plague serum on the market, which is prepared by the inoculation of dead, and later of living, cultures of the plague bacillus.

*Experiment 6. Estimation of amount of Yersin serum (prepared at the Lister Institute) necessary to neutralise 10 lethal doses of toxin.*

Rat	Serum in c.c.	Result
1	2·0	+ 1 day
2		+ "
3		+ "
4		+ "
5		+ "
6	1·0	+ "
7		+ "
8		+ "
9		+ "
10		+ "
11	0·5	+ "
12		+ "
13		+ "
14		+ "
15		+ "
16	0·1	+ "
17		+ "
18		+ "
19		+ "
20		+ "

No evidence of any antitoxic action is shown by this serum.

Similar results were obtained with two other samples of Yersin serum (Negress and Mayboy).

In the case of two other samples (Kit and Corbally) 0·5 c.c. of the serum neutralised 10 lethal doses of toxin and 0·25 c.c. serum failed to do so.

*Prophylactic value of the serum against infection.*

The serum of the horse immunised with solution *B* is capable, in common with some of the sera obtained by the usual methods of immunisation, of protecting against a subsequent inoculation of virulent plague. In experiment 7 (p. 16), the rats received the amount of serum indicated, and the standard test dose of virulent culture was given on the following day.

*Curative value in plague infection.*

So far attention has been confined to the antitoxic and anti-infectious properties of the serum, and the question remaining to be answered is:—has the serum any effect when administered to an animal that is already suffering from plague?

*Experiment 7.*

Dose of serum	Rat	Result
1·0 c.c.	{ 1	0
	{ 2	0
	{ 3	0
	{ 4	0
	{ 5	0
	{ 6	0
	{ 7	0
	{ 8	0
	{ 9	0
	{ 10	0
0·1 c.c.	{ 11	+ 4 days
	{ 12	0
	{ 13	0
	{ 14	0
	{ 15	0
	{ 16	0
	{ 17	0
	{ 18	+ 3 days
	{ 19	0
	{ 20	+ 4 days
0·01 c.c.	{ 21	+
	{ 22	+
	{ 23	+
	{ 24	+
	{ 25	+
	{ 26	+
	{ 27	0
	{ 28	+
	{ 29	+
	{ 30	0

For this purpose the following experiment was performed. Sixty rats were inoculated with the usual dose of a virulent living plague culture; ten of these were left as controls, of which seven died of plague. The remaining fifty were treated in batches of ten—five batches. Six hours after inoculation with the living culture, batches 2, 3, 4, 5 and 6 received 0·5 c.c. serum subcutaneously on the opposite side of the body to that on which the living plague culture had been inoculated. Six hours later batches 3, 4, 5, and 6 received a second and similar dose; six hours later batches 4, 5, and 6 a third; six hours later batches 5 and 6 a fourth, and six hours later batch 6 received a fifth dose.

The results of this treatment are shown in experiment 8.

This table shows:

(1) That the infectivity of the test virulent culture was well up to the standard (Vol. x. p. 544).

(2) That taking all the rats together the death rate had been reduced from 80% to 18%.

# Experiment 8.

Batch	Rat	Treatment after infection. Serum c.c.					Result	Average
		6 hrs.	12 hrs.	18 hrs.	24 hrs.	30 hrs.		
1	1	...	...	...	...	...	+ 3 days	3.5 days
	2	...	...	...	...	...	+ 3 days	
	3	...	...	...	...	...	+ 3 days	
	4	...	...	...	...	...	+ 4 days	
	5	...	...	...	...	...	+ 4 days	
	6	Controls.		No treatment.		...	+ 4 days	3.5 days
	7	...	...	...	...	...	+ 4 days	
	8	...	...	...	...	...	+ 4 days	
	9	...	...	...	...	...	0	
	10	...	...	...	...	...	0	
2	1	0.5	...	...	...	...	+ 4 days	5 days
	2	0.5	...	...	...	...	+ 6 days	
	3	0.5	...	...	...	...	0	
	4	0.5	...	...	...	...	0	
	5	0.5	...	...	...	...	0	
	6	0.5	...	...	...	...	0	5 days
	7	0.5	...	...	...	...	0	
	8	0.5	...	...	...	...	0	
	9	0.5	...	...	...	...	0	
	10	0.5	...	...	...	...	0	
3	1	0.5	0.5	...	...	...	+ 9 days	6½ days
	2	0.5	0.5	...	...	...	+ 4 days	
	3	0.5	0.5	...	...	...	0	
	4	0.5	0.5	...	...	...	0	
	5	0.5	0.5	...	...	...	0	
	6	0.5	0.5	...	...	...	0	6½ days
	7	0.5	0.5	...	...	...	0	
	8	0.5	0.5	...	...	...	0	
	9	0.5	0.5	...	...	...	0	
	10	0.5	0.5	...	...	...	0	
4	1	0.5	0.5	0.5	...	...	0	
	2	0.5	0.5	0.5	...	...	0	
	3	0.5	0.5	0.5	...	...	0	
	4	0.5	0.5	0.5	...	...	0	
	5	0.5	0.5	0.5	...	...	0	
	6	0.5	0.5	0.5	...	...	0	
	7	0.5	0.5	0.5	...	...	0	
	8	0.5	0.5	0.5	...	...	0	
	9	0.5	0.5	0.5	...	...	0	
	10	0.5	0.5	0.5	...	...	0	
5	1	0.5	0.5	0.5	0.5	...	+ 6 days	6 days
	2	0.5	0.5	0.5	0.5	...	+ 6 days	
	3	0.5	0.5	0.5	0.5	...	0	
	4	0.5	0.5	0.5	0.5	...	0	
	5	0.5	0.5	0.5	0.5	...	0	
	6	0.5	0.5	0.5	0.5	...	0	6 days
	7	0.5	0.5	0.5	0.5	...	0	
	8	0.5	0.5	0.5	0.5	...	0	
	9	0.5	0.5	0.5	0.5	...	0	
	10	0.5	0.5	0.5	0.5	...	0	
6	1	0.5	0.5	0.5	0.5	0.5	+ 9 days	10 days
	2	0.5	0.5	0.5	0.5	0.5	+ 10 days	
	3	0.5	0.5	0.5	0.5	0.5	+ 11 days	
	4	0.5	0.5	0.5	0.5	0.5	0	
	5	0.5	0.5	0.5	0.5	0.5	0	
	6	0.5	0.5	0.5	0.5	0.5	0	10 days
	7	0.5	0.5	0.5	0.5	0.5	0	
	8	0.5	0.5	0.5	0.5	0.5	0	
	9	0.5	0.5	0.5	0.5	0.5	0	
	10	0.5	0.5	0.5	0.5	0.5	0	

50 treated

9 deaths

(3) That in those cases where death of the treated rats occurred the length of life after injection was prolonged from 3–5 days to 10 days.

The next experiment was to test the maximum length of time that could be allowed to elapse between infection with plague and the inoculation of serum, consistent with the survival of the animal.

For this experiment 30 rats received the standard dose of living virulent culture. The rats were divided into three batches of 10 each. Batch 1 were not treated and remained as controls. Batch 2 received 0·5 c.c. serum 24 hours after infection. Batch 3 received 0·5 c.c. serum 48 hours after infection. The results of this experiment are shown in table 9.

*Experiment 9.*

Batch	Rat	Treatment	Days after infection										
			1	2	3	4	5	6	7	8	9	10	
2	1	0·5 c.c. serum 24 hours after infection	...	...	...	...	...	...	...	...	...	...	Survived.
	2		...	...	...	...	...	...	...	...	...	...	Survived.
	3		...	...	...	...	...	...	...	...	...	...	Survived.
	4		...	...	...	...	...	...	...	...	...	...	Survived.
	5		...	...	...	...	...	...	...	...	...	...	Survived.
	6		...	...	...	...	...	...	...	...	...	...	Survived.
	7		...	...	...	...	...	...	...	...	...	...	Survived.
	8		...	...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	...	...	+	...	...	...	...	...	Survived.
	10		...	...	...	+	...	...	...	...	...	...	Survived.
3	1	0·5 c.c. serum 48 hours after infection	...	...	+	...	...	...	...	...	...	...	Survived.
	2		...	...	...	...	...	...	...	...	...	...	Survived.
	3		...	...	+	...	...	...	...	...	...	...	Survived.
	4		...	...	...	...	...	...	...	...	...	...	Survived.
	5		...	...	+	...	...	...	...	...	...	...	Survived.
	6		...	...	+	...	...	...	...	...	...	...	Survived.
	7		...	+	...	...	...	...	...	...	...	...	Survived.
	8		...	...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	...	...	...	...	...	...	...	...	Survived.
	10		...	...	...	...	...	...	...	...	...	...	Survived.

The serum therefore saves the lives of a sensible proportion of infected rats even if its administration is delayed for as long as 24 hours after inoculation. It should be noted that the untreated rats begin to die on the second day.

In the case of the untreated batch, all the rats died; this must be taken as indicating that the virulence of the test culture was well up to the standard (80% deaths) and does not indicate any increase in virulence of the culture or diminution in resistance of the rats.

A comparative test was made between the curative value of Yersin's serum as prepared by the Lister Institute and the serum prepared by inoculation of solution B. In both cases ten rats received, 6 hours

after inoculation, 0·5 c.c. of serum hypodermically on the opposite side to the infecting dose of virulent culture. Ten control untreated rats showed the virulence of the test culture to be up to the standard.

Experiment 10.

Batch	Rat	Treatment	Result. Days after infection									
			1	2	3	4	5	6	7	8	9	10
1	1	0·5 c.c. Yersin serum 6 hours after infection	...	...	...	+						
	2		...	...	...	...	+					
	3		...	...	+							
	4		...	...	...	...	+					
	5		...	...	...	+						
	6		...	...	...	...	...	...	...	...	...	Survived.
	7		...	...	...	+						
	8		...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	+							
	10		...	...	+							
2	1	0·5 c.c. new serum 6 hours after infection	...	...	...	+						
	2		...	...	...	...	...	...	...	...	...	Survived.
	3		...	...	...	...	...	...	...	...	...	Survived.
	4		...	...	...	...	...	...	...	...	...	Survived.
	5		...	...	...	...	...	...	...	...	...	Survived.
	6		...	...	...	...	...	...	...	...	...	Survived.
	7		...	...	...	...	...	...	...	...	...	Survived.
	8		...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	...	...	...	...	...	...	...	Survived.
	10		...	...	...	...	...	+				

It has thus been demonstrated that the *antiplague serum prepared by immunising horses with a solution of the nucleo-protein from the plague bacillus prepared as described in the previous report possesses not only antitoxic and anti-infectious properties, but that it also excercises in rats a markedly curative effect when injected 24 hours after the animals have been infected with virulent plague bacilli.*

These results are most encouraging and further research is being directed towards obtaining a still more powerful serum.

# XLIII. SECOND REPORT ON INVESTIGATIONS INTO PLAGUE VACCINES.

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With 1 Diagram.

## CONTENTS.

	PAGE
I. The action of chloroform on the nucleoproteins contained in the plague bacillus . . . . .	21
1. The chloroform-nucleoprotein compound a reversible one . . . . .	22
2. Means of effecting the reversal	
A. Anhydrous sulphate of soda . . . . .	24
B. Diminished partial pressure . . . . .	24
C. Reversal effected in the body . . . . .	26
3. The action of toluol . . . . .	26
II. On the presence of a hydrolytic enzyme in the plague bacillus .	27
1. The hydrolysis of an emulsion of organisms . . . . .	28
2. The hydrolysis of the extracted nucleoproteins . . . . .	29
III. The effect of heat . . . . .	29
1. On the toxic property of the nucleoproteins . . . . .	30
2. On the hydrolytic enzyme . . . . .	31
3. Comparison of the effect of heat on the bacillus and on extracts obtained from it with regard to	
A. The toxic properties . . . . .	32
B. The immunising properties . . . . .	35
IV. The effect of hydrolysis . . . . .	35
1. On the toxic properties of the extract . . . . .	35
2. On the immunising properties of the extract . . . . .	38
V. The increase of immunising power as the result of hydrolysis	40
VI. The immunity against the toxin . . . . .	43
VII. Conclusions . . . . .	46

IN a former report (this *Journal*, Vol. x. p. 536) some of the contents of plague bacilli which had been killed by chloroform were extracted by two methods: (1) simple digestion of the organisms with saline, (2) treatment of the moist bacteria with enough anhydrous sulphate of soda to combine with all the water present, freezing and



thawing the mixture by changing the temperature from 18° C. to 37° C.<sup>1</sup>, filtering off the bacterial deposit at 37° C. and subsequent extraction of the bacteria with water.

The two solutions so obtained possess much the same chemical properties. Both contain one or more kinds of nucleoprotein, accounting for about two-thirds of the total nitrogen in the extract. As regards their physiological properties, however, there is a striking difference. The first extract, in the previous report called solution A, has only a small degree of toxicity for rats and, by the method employed (this *Journal*, Vol. x. p. 545), its immunising value was shown to be negligible. On the other hand, the second extract, obtained after treatment with sodium sulphate and called solution B, was fatal to rats, 0·05 to 0·1 mg. killing them in 18 hours: doses of 0·001 to 0·01 mg. afforded substantial protection, the latter amount reducing the mortality after the inoculation of a standard dose of plague culture from 80 % to 10 %. The toxic and immunising properties always accompanied the nucleoprotein throughout the various chemical manipulations to which it was subjected. The residue left after treatment with sodium sulphate and extracting with water was shown to be devoid of acute toxicity and immunising properties.

## I. THE ACTION OF CHLOROFORM.

The cause of the discrepancy in physiological activities of these two chemically similar extracts was not clear at the time of my previous report. I was inclined to believe that the toxic and immunising properties resided in some substance other than the nucleoprotein which for some unexplained reason was extracted together with it only after treatment with the sodium sulphate. Since then I have been able to clear up the mystery and at the same time to explain the different conclusions arrived at by Pick and myself with regard to the effect of chloroform upon the antigen of the plague bacillus.

It is stated by Pick (Kraus and Levaditi's *Handbuch*, Bd. I. p. 353) that chloroform destroys the antigen of the plague bacillus and in the first of these reports I explained that I had not been able to confirm this statement but had found that a toxic solution possessed of good immunising power could be prepared from organisms that had been

<sup>1</sup>  $\text{Na}_2\text{SO}_4$  changes to  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  between these temperatures. At 37° C. the mixture exists as an emulsion of bacteria in a saturated solution of  $\text{Na}_2\text{SO}_4$ , at 18° C. as a hard rocky mass of Glauber's salt and dry bacteria.

killed by chloroform. The explanation of these contradictory statements is afforded by the following experiments which show (1) that chloroform combines with some substance in the extract giving rise to a relatively atoxic modification; (2) that this compound is reversible; and (3) that a ready method of reversing the combination and of reinstating the toxicity is afforded by the use of anhydrous sodium sulphate.

EXP. 1. Sixty grammes of "sulphated" bacilli<sup>1</sup> were taken and divided into two portions of 30 grammes each. The sulphate was removed from both portions by adding just sufficient water to dissolve it at 37° C. and filtering. The pasty masses of bacilli left on the filter were treated as follows:

part 1 was extracted for 3 hours in chloroform water,

part 2 was extracted for 3 hours in distilled water: both at 37° C.

Both portions were then filtered and the nucleoprotein in the filtrate determined as usual. Both extracts contained the same quantity of nucleoprotein. The lethal dose of the nucleoprotein of the first solution was 2 mg., of the second 0.1 mg. The chloroform water extract although containing the same amount of nucleoprotein only possessed  $\frac{1}{20}$ th the toxicity of the water extract. This might be explained on the assumption that the nucleoprotein was not the active agent and the following experiment supports this interpretation.

EXP. 2. The same experiment was repeated with the modification of adding chloroform to the water extract after the completion of the extraction. The results are given below and show that the toxicity of the solution was unaltered. The degrading effect of chloroform on toxicity is thus greatest at the moment of extraction.

Details of experiments 1 and 2 are given in Table I.

In my previous report it was shown that the toxic and immunising properties of the extract prepared by the "sulphate process" followed so closely the content of nucleoprotein that it was difficult to believe that they belonged to some merely adherent substance and not to the nucleoprotein itself.

The following experiments, which show that the action of chloroform

<sup>1</sup> Wherever possible, in the course of this work, the various extracts have been made from the same batch of "sulphated" bacilli, a large stock of which, several kilogrammes, was prepared, thoroughly mixed and kept in the ice box. The method of preparation of this powder of "sulphated" bacilli is given in the last report in full. Briefly it is the washed surface growth from agar set in Roux flasks, mixed with anhydrous sulphate of soda and ground to a fine powder. Once prepared it apparently preserves its properties indefinitely and is a most convenient means of ensuring a constant supply of plague bacilli.

on the nucleoprotein is reversible, completely explain the apparent inconsistency of the results of experiments 1 and 2 with this view.

EXP. 3. A quantity of an emulsion of organisms was prepared and a few cubic centimetres of chloroform added to the flask so that some remained free and undissolved at the bottom of the flask. The emulsion

TABLE I. *Experiment 1.*

<i>Toxicity of the extract made in chloroform water.</i>			<i>Toxicity of the water extract.</i>		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
1·7	{ 1	* + 1 day	1·7	{ 1	+ 1 day
	{ 2	0		{ 2	+ 1 day
0·8	{ 3	0	0·8	{ 3	+ 1 day
	{ 4	0		{ 4	+ 1 day
0·4	{ 5	0	0·4	{ 5	+ 1 day
	{ 6	0		{ 6	+ 1 day
0·2	{ 7	0	0·2	{ 7	+ 1 day
	{ 8	0		{ 8	+ 1 day

\* The symbol + is used throughout this report to indicate the death of the animal, 0 its survival.

*Experiment 2. Toxicity of the water extract to which chloroform was added.*

Dose, mgs.	Rat	Result
1·7	{ 1	+ 1 day
	{ 2	+ 1 day
0·8	{ 3	+ 1 day
	{ 4	+ 1 day
0·4	{ 5	+ 1 day
	{ 6	+ 2 days
0·2	{ 7	+ 2 days
	{ 8	—

TABLE II.

*Experiment 3. Determination of toxicity.*

Dose, mgs.	(1) Emulsion in chloroform water		(2) Same emulsion after pumping out the chloroform	
	Rat	Result	Rat	Result
1·0	{ 1	0	1	+ 1 day
	{ 2	0	2	+ 1 day
0·8	{ 3	0	3	+ 1 day
	{ 4	0	4	+ 1 day
0·6	{ 5	0	5	+ 1 day
	{ 6	0	6	+ 1 day
0·4	{ 7	0	7	+ 1 day
	{ 8	0	8	+ 1 day
0·2	{ 9	0	9	+ 1 day
	{ 10	0	10	+ 1 day

was divided into two parts. Both parts were incubated at 37° C. for 6 hours. Part 1 was set aside, while part 2 was exhausted by the Fleuss pump for some hours until no smell of chloroform was noticeable. Both parts were left till the next day when they were filtered and the nucleoprotein content and toxicity determined (see Table II).

The lethal dose of the pumped out portion was 0.2 mg., possibly less, while that from which the chloroform had not been so removed was greater than 1 mg. The amount of nucleoprotein was the same in each.

EXP. 4. A quantity of organisms killed by chloroform were mixed with sulphate of soda in the usual way and the sulphate removed by dissolving with water at 37° C. As we have seen, this process yields organisms free from chloroform and in such a condition that the nucleoprotein with its toxic and antigenic properties can be extracted with water from the bacilli. In this case chloroform was added to the water with which the paste of organisms was extracted. The chloroform water extract was comparatively toxic, although it contained the usual amount of nucleoprotein. On pumping out the chloroform, however, the solution was again rendered toxic to the usual degree (M.L.D. = 0.1 mg.). After this extraction the organisms were again mixed with sulphate which in its turn was removed. The paste of organisms left after this second sulphating was extracted in water and a small yield of nucleoprotein again obtained, of which the lethal dose was as usual about 0.1 mg. (see Table III).

These experiments show that the atoxic nucleoprotein extracted by means of chloroform water can be rendered toxic by the simple process of removing the chloroform in a vacuum. There is, therefore, no reason to suppose, as was done in my previous report, that the nucleoprotein extracted by water from the organisms killed by chloroform (nucleoprotein A in the last report) is not the same substance as that extracted from similar material after sulphating (nucleoprotein B of last report). The nucleoprotein A is in combination with chloroform, and for this reason has lost its toxic properties which, however, can be reinstated by removing the associated chloroform with the air pump. It is further clear that one of the effects of the sulphate of soda used in the process is to remove this chloroform from its loose combination with the nucleoprotein.

This removal of chloroform by the sulphate of soda appears to be due to evaporation of the chloroform following on the withdrawal of all the water by the anhydrous salt to form  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ .

TABLE III. *Toxicity determinations.*

Dose mgs.	Chloroform water extract		Chloroform water extract after pumping out the chloroform	
	Rat	Result	Rat	Result
2.5	{ 1	0	1	+ 1 day
	{ 2	0	2	+ 1 day
1.25	{ 3	0	3	+ 1 day
	{ 4	0	4	+ 1 day
0.5	{ 5	0	5	+ 1 day
	{ 6	0	6	+ 1 day
0.25	{ 7	0	7	+ 1 day
	{ 8	0	8	+ 1 day

Dose, mgs.	Second water extract	
	Rat	Result
1.1	{ 1	+ 1 day
	{ 2	+ 1 day
0.56	{ 3	+ 1 day
	{ 4	+ 1 day
0.28	{ 5	+ 1 day
	{ 6	+ 2 days
0.05	{ 7	+ 3 days
	{ 8	+ 3 days

Why the effect of chloroform upon the toxicity and immunising power of the nucleoproteins should be so different, according to whether it is brought into play at the moment of extraction or subsequently (as in Experiment 1), still remains unexplained. It may well be that the composition of these nucleoproteins at the moment of soaking out from the recently killed bacillus is somewhat different to that which obtains afterwards. Nucleoproteins are bodies with great possibilities of combination with other proteins and there is no evidence that they exist as such inside the living cell.

An interesting point with regard to the reversibility of the chloroform-nucleoprotein-toxin combination emerges from the study

TABLE IV.

*Delayed mortality, using chloroformed nucleoprotein.*

Dose, mgs.	Rat	Result
1.6	{ 1	+ 1 day
	{ 2	0
0.83	{ 3	+ 8 days
	{ 4	+ 8 days
0.4	{ 5	+ 5 days
	{ 6	+ 4 days
0.2	{ 7	+ 27 days
	{ 8	+ 10 days

of the effect of this substance when inoculated into rats. The lethal dose (in this work the death of the animal in two days is regarded as determining the L.D.) is large (1 to 2 mg.) but there is observed a delayed mortality amongst the animals corresponding to a gradual dissociation of the compound after inoculation. Thus in one experiment the results in Table IV were obtained.

It will be a matter of interest to follow up this delayed toxic action in the body since its bearing on the problem of vaccination is apparent.

*The effect of toluol on the toxicity and immunising properties of the nucleoprotein.*

Toluol exerts but slight deleterious influence upon the activity of solutions of the nucleoproteins of the plague bacillus and was found to be the best preservative to use during their preparation. I tried therefore whether this substance could be advantageously employed instead of chloroform vapour to kill the bacilli.

Preliminary experiments were made to determine the time necessary to kill the organisms. For this purpose a good surface growth on agar in a Roux flask was used. The flask was inverted and a few cubic centimetres of toluol introduced. At the end of each quarter of an hour a whole loopful of the growth was withdrawn from the flask and inoculated on the surface of agar. In this way the following results (Table V) were obtained :

TABLE V.

*Showing the presence or absence of growth in the tubes inoculated from the flask after exposure to toluol vapour for the times indicated.*

Quarter hour intervals	Growth after		
	24 hours	48 hours	72 hours
1	+	+	+
2	+	+	+
3	+	+	+
4	-	+	+
5	-	+	+
6	-	+	+
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-
11	-	-	-

These preliminary experiments showed that toluol can be used as a germicide in the same way as chloroform (viz. inverting the Roux flask and introducing a few c.c. of the liquid into the flask, thus exposing the bacilli to the action of the vapour only), about one and a half hours being required to completely sterilize a Roux flask. Seventy-three Roux flasks were treated in this way. Of the emulsion obtained, one half was left in contact with toluol for two days, the other half was centrifugalised and washed as usual. After washing, the latter was sulphated in the ordinary way, and a water extract corresponding to solution B obtained; of this solution 0·2 mg. nucleoprotein was a lethal dose. The portion of the emulsion left in contact with the toluol was filtered, and of the fluid obtained the lethal dose was about 1·0 mg.

Toluol apparently behaves similarly to chloroform and forms a loose combination with the nucleoprotein, the combination being less toxic. By treatment with anhydrous sulphate of soda a more toxic product is obtained (Table VI).

TABLE VI.

<i>Toxicity of toluol water extract.</i>			<i>Toxicity of water extract from toluol-killed and sulphated organisms.</i>		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
1·5	{ 1	+ 3 days	4·0	{ 1	+ 1 day
	{ 2	0		{ 2	+ 1 day
0·6	{ 3	+ 3 days	2·0	{ 3	+ 1 day
	{ 4	0		{ 4	+ 1 day
0·3	{ 5	+ 3 days	1·0	{ 5	+ 1 day
	{ 6	0		{ 6	+ 1 day
0·15	{ 7	0	0·4	{ 7	+ 1 day
	{ 8	0		{ 8	+ 1 day
			0·2	{ 9	+ 1 day
				{ 10	+ 2 days

## II. ON THE PRESENCE OF A HYDROLYTIC ENZYME IN THE PLAGUE BACILLUS.

A quantity of plague bacilli were grown on agar in Roux flasks. They were killed by chloroform vapour and an emulsion made in salt solution: 8 % of the nitrogen of the emulsion was soluble in saturated tannic acid<sup>1</sup>. The emulsion was incubated under toluol at 37° C.

<sup>1</sup> This method of determining the progress of hydrolysis is due to Hedin (Hedin and Rowland, "Ueber ein Proteolitisches Enzym in der Milz," *Zeitschr. f. phys. Chem.* Bd. xxxii. p. 342). The precipitation of the undigested proteids is effected by the addition of an equal volume of a solution of tannic acid 7 % and acetic acid 5 %.

After 24 hours 28 % of the total nitrogen was in a form which was not precipitated by tannic acid. In 6 days 38 % was reduced to this form, while in 10 days 50 % was so changed.

The experiment was repeated with the addition of 11 % sulphate of soda to the emulsion. In this case 38 % of the nitrogen was converted in 24 hours and 48 % after 10 days.

The solution contained 780 milligrammes of protein in 100 c.c. The quantities found after the lapse of the times indicated are given in Table VII.

TABLE VII.

Time	Protein found, mgs. per 10 c.c.
As prepared	78
18 hours	68
45 hours	49
65 hours	49
22 days	38

The diminution of the nitrogen precipitable by tannic acid and of the total proteins coagulable by heat runs parallel, from which it is concluded that the bulk of the protein hydrolysed by the enzyme is derived from the nucleoprotein. The great falling off in rate as the hydrolysis proceeds at the stage when less than 50 % of the total protein has been attacked, suggests either that the enzyme is readily destroyed, or that only a proportion of the protein is capable of hydrolysis by this means. The effect of the hydrolysis by this enzyme upon the toxicity and immunising power of the nucleoprotein extract will be dealt with directly (p. 35 below).

There exists, therefore, in the plague bacillus an enzyme capable of hydrolysing the protein. For the sake of comparison with a phenomenon which is better known, an experiment was made with some brewer's yeast under the same conditions. Here after 5 days 73 % of the nitrogen was converted, while in the presence of sulphate of soda (10 %) 59 % was changed.

The activity of this enzyme is therefore comparable with that of a proteolytic ferment of recognised power.

The question now arises, is this enzyme extracted by the methods employed in preparing an actively immunising solution by the sulphate process? A quantity of solution of nucleoproteins was prepared by dehydration with  $\text{Na}_2\text{SO}_4$  and extraction in saline and examined in the same way. The percentage of nitrogen hydrolysed to the extent of being soluble in tannic acid mixture is shown in Table VIII.



TABLE VIII.

*The solution contained 7·8 mgs. of nucleoprotein per c.c.*

Time	Percent. nitrogen not precipitable by tannic acid mixture after various intervals at 37° C.
As prepared	5·7
18 hours	29
45 hours	41·7
65 hours	44
22 days	56

Concurrently with this hydrolysis there was a loss in protein as estimated by boiling after acidulation with acetic acid.

### III. THE EFFECT OF HEATING SOLUTIONS OF THE NUCLEOPROTEIN ON THEIR IMMUNISING POWER, TOXICITY AND THE ACTIVITY OF THE HYDROLYTIC ENZYME CONTAINED THEREIN.

As shown by Famulener and Madsen (*Biochem. Zeitschr.* Bd. XI. p. 186, 1908), and by Martin and Chick (*Journ. Physiol.* Vol. XL. p. 404, 1910), the effect of hot water upon certain antigens (vibrio-lysin, tetano-lysin) and upon proteins does not occur instantaneously when a particular temperature is reached. It is a time process in which heat merely plays the subsidiary part of an accelerator. The action has an extraordinary high temperature coefficient and the rate of the reaction is increased in some cases as much as twice per degree centigrade rise in temperature.

Famulener and Madsen studied the effect of hot water upon vibrio-lysin and tetano-lysin and were able to obtain data for the calculations of the antigen destroyed as time elapsed by test tube experiments.

In the case of plague antigen, however, *in vitro* experiments are not feasible and the number of animal experiments required to determine each point would be very costly. I have therefore been obliged to content myself with observations indicating the fall in antigenic power suffered by the minimal optimal immunising dose by heating for half an hour to various temperatures. The diminution in immunity conferred was indicated by the percentage of animals which survived a subsequent inoculation of 0·1 c.c. of the standard plague culture after they had received the same quantity of antigen solution heated at each temperature. What the relation between the percentage protected and

proportion of antigen remaining after heating may be, is at present being determined.

As stated in my previous report (this *Journal*, Vol. x. p. 559), the protection afforded by a single dose of one-hundredth of a milligramme of the nucleoprotein and associated antigen is to protect from 80–90 % of the rats inoculated against a subsequent dose of virulent plague such as will kill 80 % of the control animals. The following table gives the protection afforded by a similar dose of nucleoprotein which had been heated to the temperatures named for half an hour.

TABLE IX.

Temp. to which antigen solution was heated (°C.)	No. of rats in series	Mortality	% Mortality
Unheated	19	2	11
55	20	6	30
60	36	18	50
64	39	21	54
65	38	30	79
Controls inoculated with test culture only	100	—	78

In the last series a precipitation took place which was filtered off, the clear fluid only being inoculated.

This experiment shows that the destruction of antigen is appreciable below 55° C. and that the rate increases slowly until 64° C. is reached. At 65°, at which temperature the nucleoprotein was precipitated<sup>1</sup>, the whole of the antigen disappeared from the solution, for the mortality was the same as in the controls.

1. *The effect of heating upon the toxicity of solutions of nucleoprotein.*

A quantity of toxic nucleoprotein solution was prepared and heated in the water bath to various temperatures for half an hour. Table X below shows the experimental results of inoculating these heated solutions into rats.

The minimal fatal dose of this sample of the nucleoprotein was evidently just above 0.1 mg., for this quantity of the unheated material killed the two rats in two and three days respectively. Heating to 53° C. and 60° C. for half an hour reduced the toxicity to  $\frac{1}{4}$ th (0.4 mg. = M.L.D.) and  $\frac{1}{9}$ th (0.8 to 1 mg. = M.L.D.) respectively. The observations at 56° C. only give an upper limit.

<sup>1</sup> The precipitation of the nucleoprotein by heat does not always occur at this temperature. The exact point is dependent upon the reaction and concentration of the solution.

From the series at 53° C. and at 60° C. some idea of the acceleration of the destruction per degree rise of temperature can be obtained. Taking 0·4 mg. as the minimal lethal dose after heating to 53° C. for half an hour and 0·9 mg. as the mean value after heating to 60° C. for the same period, the relative rates of destruction are as 9 to 4 for a rise of 7° C. This gives a temperature coefficient of 3·2 per 10° C.

TABLE X.

The solutions were of the same reaction and nucleoprotein concentration and were heated to the temperatures for half an hour.

Dose, mgs.	Rat	Kept in ice box	53° C.	56° C.	60° C.
4·0	{ 1	...	...	+ 1 day	...
	{ 2	...	...	...	+ 3 days
2·0	{ 3	...	+ 1 day	+ 1 day	...
	{ 4	...	+ 1 day	+ 1 day	...
1·0	{ 5	...	+ 1 day	+ 1 day	+ 3 days
	{ 6	...	+ 1 day	+ 3 days	0
0·8	{ 7	...	+ 1 day	...	+ 3 days
	{ 8	...	0	...	0
0·6	{ 9	+ 1 day	+ 1 day	+ 1 day	0
	{ 10	+ 1 day	+ 1 day	+ 1 day	0
0·4	{ 11	+ 1 day	+ 1 day	...	0
	{ 12	+ 1 day	0	...	0
0·2	{ 13	Very ill	...	...	...
	{ 14	Very ill	...	...	...
0·1	{ 15	+ 2 days	...	...	...
	{ 16	+ 3 days	...	...	...

## 2. *The effect of heating solutions of the nucleoprotein upon their hydrolytic activity.*

Like other enzymes, the hydrolytic ferment in the plague bacillus is destroyed by heating its solution. It is impossible to properly determine the influence of temperature upon the rate of the destruction of the enzyme by hot water, because, so far, I have not succeeded in separating the enzyme from the "substrate." Some qualitative notion of the influence of hot water was, however, obtained by heating solutions of the nucleoprotein containing the enzyme for half an hour at various temperatures, and estimating the amount of non-precipitable nitrogen after two days' subsequent incubation at 36° C. (Table XI).

As this method does not give an estimate of the work done by the enzyme after heating irrespective of the increased work done by the enzyme during the heating, inasmuch as it is not possible to instantaneously bring the solution to the temperature required, the experiment

was repeated with a series of controls in which the changed nitrogen was estimated immediately after the heating. The results are given in Table XII.

TABLE XI.

Temp. to which the solution was heated	Percent. nitrogen not precipitated by tannic acid mixture
Control (not heated)	36.8
51—52° C.	33.8
53—54	30.4
55—56	24.5
59—60	16.6
63—64	13.0

TABLE XII.

Temp. to which the solution was heated for 30 mins.	Amount of nitrogen in non-precipitable form	
	Determined at once	After two days
Control	10.9 %	49.5 %
39° C.	12.1	—
44	—	47.9
50	14.7	—
55	15.0	40.0
58	15.1	—
63.5	13.1	17.4

These determinations indicate that on heating for half an hour the temperature at which the first damage to the enzyme can be recognised is about 50° C.

3. *Comparison of the effect of heating the bacilli on the toxicity and immunising power of the extract subsequently obtained from them, with the effect of heat applied to the extract itself.*

A. *With regard to toxicity.*

For this experiment 100 Roux flasks of plague bacilli were prepared as usual, but instead of being chloroformed they were heated to 60° C. in the steamer. The steamer was first raised to this temperature, then opened, the flasks introduced and when it had again reached 60° C. the time was noted and heating continued for half an hour. The surface growth was washed off as usual in salt solution and the emulsion centrifugalised. The first washings, *i.e.* the salt solution that had been in contact with the bacilli for some two or three hours, in contrast to what occurred in the case of bacilli killed by chloroform, contained no

nucleoprotein in solution. The paste of organisms was "sulphated" in the usual way, the mass left to cool and placed at 37° C. for the night. Next morning enough water at 33° C. to dissolve the sulphate was added and the whole placed on a hardened filter. The residue of organisms left on the filter was extracted with water. The suspension would not filter and rapidly became a slimy mass resembling sputum. It was accordingly centrifuged and an opalescent fluid obtained. The amount of nucleoprotein obtained was equal to that usually yielded by similar treatment of chloroform killed, *i.e.* unheated, bacilli. The nucleoprotein obtained by this process was of about the same toxicity as that obtained after killing the bacilli with chloroform, 0.2 mg. being an acute lethal dose.

In view of the effects of heating the toxin already given, the fact that this solution was highly toxic indicates that there is a considerable difference in the effect produced by heating it in solution in water and heating it in the condition in which it occurs within the body of the bacillus.

TABLE XIII.

<i>Lethal dose of extracted nucleoprotein heated to 55° C. for half an hour.</i>			<i>Lethal dose of an emulsion heated to 55° C. for half an hour.</i>		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
2.0	{ 1	+ 1 day	2.0	{ 1	+ 1 day
	{ 2	+ 1 day		{ 2	+ 2 days
1.0	{ 3	+ 1 day	1.0	{ 3	+ 1 day
	{ 4	+ 2 days		{ 4	+ 3 days
0.6	{ 5	+ 1 day	0.5	{ 5	0
	{ 6	+ 1 day		{ 6	0
0.4	{ 7	0			
	{ 8	0			

The difference between the effect of heat upon the toxicity of the extracted nucleoprotein and of the bacillus respectively was further studied by comparing the toxicity of the extracted nucleoprotein heated to 55° C. with that of organisms heated to the same temperature.

In order to institute a comparison between the toxicity of the whole bacilli and that of the extracted nucleoprotein it is necessary to know what proportion of the bacillus the latter comprises. From previous observations (this *Journal*, Vol. x. p. 536) it was found that the nucleoprotein constitutes about one-tenth by weight of the whole bacillus. The toxicity of the extracted nucleoprotein solution after heating to 55° C. for half an hour was found by experiment to be between 0.5 and

1 mg. On the assumption that the effect of heat upon toxicity when the bacillus is heated as a whole is the same as when the toxic nucleoprotein is heated after extraction, the lethal dose of whole bacilli heated to 55° C. should be 6.0 mg. whereas it was about 1 mg. The protocols of this experiment are given in Table XIII.

The contrast is still more marked if a comparison be made between the toxicity of the nucleoprotein solution made from unheated organisms and a similar solution made from organisms that have been heated to 60° C. for half an hour, a temperature which we have seen reduces the toxicity of the nucleoprotein solution to one-ninth. At a temperature at which the toxicity of a solution in water is greatly reduced, only a small diminution occurs when the toxic nucleoprotein is in the cell, as the following experiment shows.

TABLE XIV.

<i>Toxicity of nucleoprotein solution heated to 60° C. for half an hour.</i>			<i>Toxicity of nucleoprotein solution prepared from organisms that had been heated to 60° C. for half an hour.</i>		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
4.0	{ 1	+ 5 days	0.8	{ 1	+ 1 day
	{ 2	0		{ 2	+ 1 day
1.0	{ 3	+ 3 days	0.4	{ 3	+ 1 day
	{ 4	0		{ 4	+ 2 days
0.8	{ 5	+ 3 days	0.2	{ 5	+ 2 days
	{ 6	0		{ 6	+ 2 days
0.6	{ 7	0	0.1	{ 7	0
	{ 8	0		{ 8	0

The lethal dose of the toxin prepared from unheated organisms is about 0.1 mg.

I have at present no explanation to offer to account for the difference in the rate of destruction of the toxin according to whether it is heated whilst within the bacillus or after extraction. The very large effect of small changes in the concentration of hydrogen ions and variations in the concentration of salts upon the rate with which proteins are attacked by hot water recently described by Chick and Martin, *Journal of Physiol.* Vol. XL. p. 404, 1910, suggests a possible explanation. This subject is at present under investigation, because of the practical importance of any facts which may lead to a differential method of obtaining the antigen and toxin which are apparently bound up in the nucleoprotein.

B. *With regard to immunising power.*

A comparison of the effect of heat upon the immunising properties of the nucleoprotein (1) whilst within the bacillus and (2) subsequent to extraction, indicates that under the former circumstances a similar diminution in rate of destruction occurs to that found in the case of toxicity.

An emulsion of organisms was prepared and killed by heating to 55° C. for half an hour. Of 34 rats that had received such a volume of this emulsion as contained 0.01 mg. of dry bacterial substance, 31 survived the subsequent test inoculation with plague. The amount of nucleoprotein contained in the quantity of organisms inoculated was (as was shown in the previous report, this *Journal*, Vol. x. p. 536) about one-tenth by weight, or 0.001 mg.

A second series of 20 rats were inoculated with ten times this quantity of extracted nucleoprotein that had been heated to 55° C. for half an hour precisely as was the emulsion used in the last experiment. Of these rats only 14 survived the subsequent test inoculation. There is here an increase of 21 per cent. of survivals in the case where the antigen had been heated inside the bacillus; and this increase in face of the fact that the dose of antigen was one-tenth as great<sup>1</sup>.

IV. THE EFFECT OF HYDROLYSIS ON THE TOXICITY AND IMMUNISING PROPERTIES OF THE NUCLEOPROTEIN SOLUTION.

1. *Effect on toxicity.*

As mentioned on p. 27 above the watery or dilute saline extract of plague bacilli killed by chloroform vapour contains a hydrolytic enzyme. It had been noticed in the course of this investigation that the toxicity of an extract diminished day by day when kept at laboratory temperatures and in view of the fact recorded by Ruffer and Wilmore<sup>2</sup> that the endotoxin obtained from the dysentery bacillus is destroyed by digestion with pepsin, it was considered likely that this might be accounted for by the action of the enzyme. The following experiment shows that the

<sup>1</sup> That it was really one-tenth is a deduction based on the nucleoprotein content of the two vaccines. Until the relation between antigen injected and survivals has been worked out so as to afford a biological test for the amount of antigen, this is the only quantitative method of indicating the amount of antigen we possess.

<sup>2</sup> *British Medical Journal*, 1908, Vol. II. p. 1176.

progressive hydrolysis of the nucleoprotein on incubating the solution at 37° C., described on p. 28 above, is accompanied by a loss of toxicity. This is shown in Tables XV and XVI below. The extract was slightly alkaline to litmus paper.

TABLE XV.

The original solution contained 7·8 mg. of nucleoprotein per cubic centimetre.

Time incubated at 37° C.	Lethal dose*
Fresh	0·05 c.c.
18 hours	about 0·4
45 hours	more than 1·0
65 hours	„ 7·0

\* Portions removed from time to time to test toxicity were diluted 1 in 7·8.

TABLE XVI.

*Protocols for determination of toxicity experiment in Table XV above.*

Fresh solution			Solution incubated for 18 hrs.			Solution incubated for 45 hrs.		
Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result
0·3	{1	+ 1 day	0·4	{1	+ 1 day	1·0	{1	0
	{2	+ 2 days		{2	0		{2	0
0·2	{3	+ 1 day	0·3	{3	+ 2 days	0·8	{3	0
	{4	+ 2 days		{4	0		{4	0
0·1	{5	+ 1 day	0·2	{5	0	0·6	{5	+ 1 day
	{6	0		{6	0		{6	0
0·05	{7	+ 1 day	0·1	{7	0	0·4	{7	0
	{8	+ 2 days		{8	0		{8	0

Other determinations of the same point are here given (Tables XVII–XX). In these cases the solutions were kept at the temperature of the laboratory.

TABLE XVII.

The original solution contained 3·5 mg. of nucleoprotein per cubic centimetre.

Age	Lethal dose*
1 day	0·1 c.c.
24 days	1·0
54 days	more than 2·0
72 days	about 7·0

\* Portions removed from time to time to test toxicity were diluted 1 in 3·5.



TABLE XVIII.

*Protocols of experiments in determination of toxicity in Table XII above.*

Fresh solution.			Solution incubated for 24 days.			Solution incubated for 54 days.		
Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result
0.6	{ 1	+ 1 day	4.0	{ 1	+ 1 day	7.0	{ 1	+ 1 day
	{ 2	+ 1 day		{ 2	+ 1 day		{ 2	+ 1 day
0.4	{ 3	+ 1 day	2.0	{ 3	+ 1 day	3.5	{ 3	+ 2 days
	{ 4	+ 2 days		{ 4	+ 1 day		{ 4	+ 2 days
0.2	{ 5	+ 1 day	1.0	{ 5	+ 1 day			
	{ 6	+ 2 days		{ 6	+ 1 day			
0.1	{ 7	+ 2 days	0.6	{ 7	0			
	{ 8	0		{ 8	0			
			0.4	{ 9	0			
				{ 10	0			
			0.2	{ 11	0			
				{ 12	0			
			0.1	{ 13	0			
				{ 14	0			

TABLE XIX.

The original solution contained 5 mg. of nucleoprotein per cubic centimetre.

Age	Lethal dose *
Fresh	0.1 c.c.
4 days	0.4
17 days	about 4.0

\* Portions removed from time to time to test toxicity were diluted 1 in 5.

TABLE XX.

*Protocols of experiments in determination of toxicity, in Table XIII above.*

Fresh solution.			Solution incubated for 4 days.			Solution incubated for 17 days.		
Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result
0.4	{ 1	+ 1 day	0.4	{ 1	+ 2 days	0.6	{ 1	+ 2 days
	{ 2	+ 1 day		{ 2	0		{ 2	0
0.3	{ 3	+ 1 day	0.3	{ 3	+ 3 days	0.4	{ 3	+ 2 days
	{ 4	+ 1 day		{ 4	0		{ 4	+ 2 days
0.2	{ 5	+ 1 day	0.2	{ 5	0	0.2	{ 5	0
	{ 6	+ 2 days		{ 6	0		{ 6	0
0.1	{ 7	+ 2 days	0.1	{ 7	0	0.1	{ 7	0
	{ 8	0		{ 8	0		{ 8	0
0.05	{ 9	0						
	{ 10	0						

The accurate determination of the lethal dose of toxin demands a large number of animals. The results obtained in these three cases are sufficient to establish the fact that on hydrolysis there is a progressive loss of toxicity but are not sufficient to effect correlation between the extent of the loss and the work done by the ferment.

## 2. *Effect on the immunising power.*

As in the previous series of experiments the hydrolytic agent was the enzyme naturally occurring in the extract containing the nucleoprotein. The extract contained 8 milligrammes of nucleoprotein per cubic centimetre, and possessed a faintly alkaline reaction to litmus paper. It was kept at a temperature of 37° C. The amount of hydrolysis taking place in such an extract so kept and its effect upon the toxicity of nucleoprotein have already been considered (*vide* pp. 28, 36 above). We have now to determine what is the effect of the hydrolysis of the solution upon its immunising property.

In the first report (*vide* this *Journal*, 1910, Vol. x. p. 559), it was shown that a dose of extract containing 0·01 mg. nucleoprotein immunised from 80–90 % of rats against infection by a standard dose of the virulent living culture.

The source of this extract was an emulsion of organisms killed by chloroform vapour. We now know that during the time of exposure to chloroform, hydrolysis is proceeding, for it has been shown that it takes place in a chloroform water suspension. In dealing with the relation between hydrolysis and immunising power, it therefore follows that account must be taken of the influence of the time factor in the method of preparation.

As a preliminary an extract was prepared according to the original method which may be considered to have been hydrolysing for one day. The results of its inoculation into rats confirmed those obtained a year before, that a volume containing 0·01 mg. nucleoprotein protects 80–90 % the vaccinated animals.

TABLE XXI.

*Protection afforded by extract hydrolysed for one day.*

Dose, mg.	No. of rats inoculated	No. survived	Percentage surviving
0·01	19	17	89

Preliminary trials having indicated that the effect of hydrolysis on the immunising properties of the extract was small it was allowed to autolyse at 37°C. for upwards of two months. After one month 14 rats were inoculated with a volume which contained 0.01 mg. of nucleoprotein at the beginning of this lengthy incubation. Of these animals 12 survived the subsequent test dose, *i.e.* 83 %. It follows then that the lysis of the nucleoprotein far from having destroyed its immunising power, had left it unaltered. Referring to what has already been said in this report as to the effect of lysis, it will be remembered that at this stage of the process some half of the available nitrogen has been transformed to a condition not precipitable by tannic acid mixture.

Having then failed to find that after one month's autolysis there was any diminution in the immunity conferred by the extract, corresponding to the reduction of toxicity, it was returned to the incubator and hydrolysis allowed to continue for a further period of six weeks. At the end of this time 15 rats received the same dose as before and of these 11 survived the subsequent standard infecting dose of living culture, giving a protection of 73 %.

The diminished percentage of protections may indicate that at this late stage of hydrolysis the immunising value of antigen is beginning to be affected, but in a series of 15 rats it is hardly outside the statistical error.

We are thus obliged to admit that the protection afforded by a solution of the nucleoprotein that was originally toxic is but slightly, if at all, diminished by allowing the solution to hydrolyse whereas this process has been shown to destroy the toxicity of the solution.

In addition to the experiments just mentioned where after one month and two and a half months the animals received of the autolysed extract doses whose volumes corresponded to 0.01 mg. of the original nucleoprotein content, other series of rats were inoculated with one-tenth this quantity of extract. Thus of 15 rats that received the dose equivalent to 0.001 mg. of the one month's hydrolysed extract, eight survived the subsequent test inoculation (53 %), and of 15 rats that received the dose equivalent to 0.001 mg. of the two and a half months' hydrolysed extract seven survived the test inoculation (46 %). Reducing the dose of the one month's hydrolysed extract to one-tenth thus reduced the percentage protection from 86 to 53 and reducing the dose of the two and a half months' hydrolysed extract to the same amount reduced the percentage protection from 73 to 46.

In both cases the same reduction of the dose effected approximately the same reduction in the percentage protection.

This result affords corroborative evidence as to the relative accuracy of the percentages given by the experiment and used as a measure of the amount of immunising substance in the extract.

Putting these results in the form of a table we have :

TABLE XXII.

*Effect of hydrolysis on immunising substance.*

Time of hydrolysis	Dose, mg. nucleoprotein	No. of rats inoculated	No. of rats surviving	Percentage surviving
1 day	0.01	19	17	89
1 month	{ 0.01	14	12	83
	{ 0.001	15	8	53
2½ months	{ 0.01	15	11	73
	{ 0.001	15	7	46

The effect of hydrolysis under the influence of the hydrolytic enzyme normally present in extracts of the plague bacillus upon the immunising power presents analogies with Ruffer and Wilmore's (1908) results upon the hydrolysis of the endotoxin of the dysentery bacillus<sup>1</sup>. These authors found that by digesting for three days with pepsin and hydrochloric acid the toxicity was reduced to  $\frac{1}{30}$ th whilst the immunising value was not greatly, if at all, diminished. Gottstein (1908) also digested an emulsion of typhoid bacilli with pepsin and obtained a soluble product which he called "fermo toxin." This solution was toxic for guinea pigs and possessed of immunising action which according to this author was not impaired by boiling<sup>2</sup>.

#### V. THE INCREASE OF IMMUNISING POWER AS THE RESULT OF HYDROLYSIS.

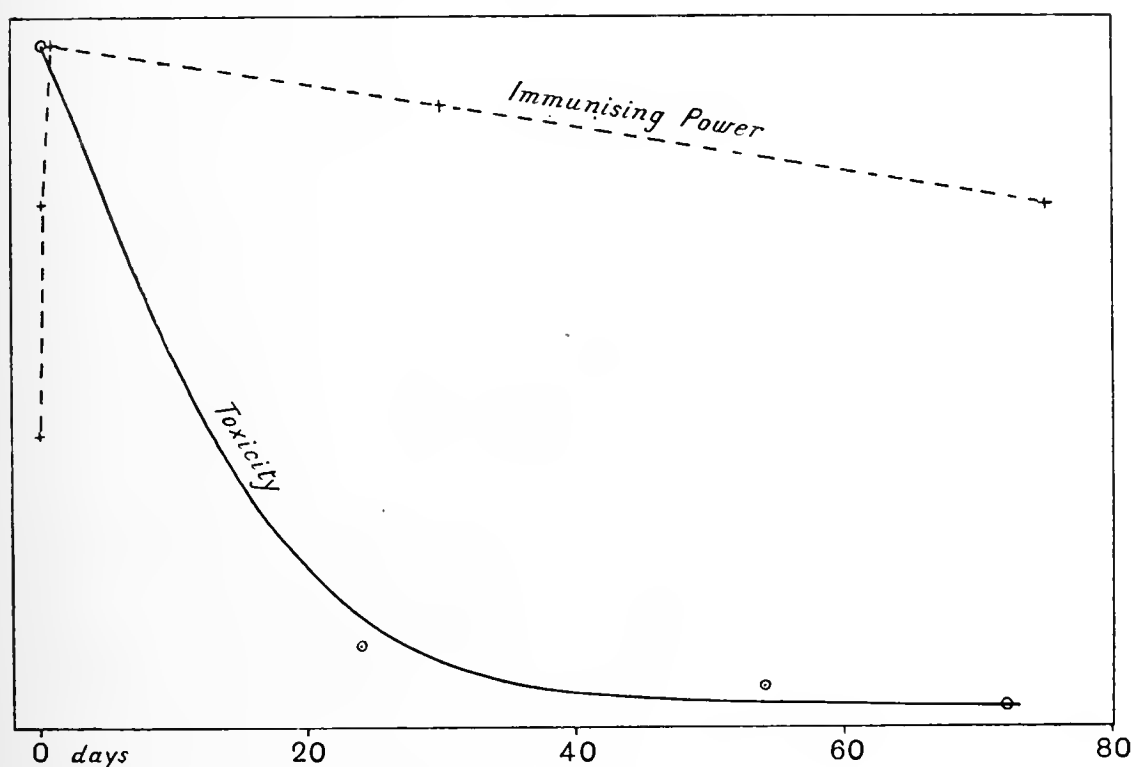
The extract prepared by the method detailed in the last report has been considered as having been subjected to hydrolysis during the time occupied in the preparation. Roughly we may consider such an extract to have been hydrolysed for one day. When it is remembered that the rate of hydrolysis is greatest in the earlier stages, the importance of being able to compare the effect upon the toxicity and immunising value in these early stages becomes obvious.

<sup>1</sup> *British Medical Journal*, 1908, Vol. II. p. 1176.

<sup>2</sup> *Deutsches Arch. f. klin. Med.* Vol. xciv. p. 255.

In the method as originally described the bacilli grown on the surface of agar in Roux flasks were submitted to the action of chloroform vapour for 12 hours; during the whole of this time, as we now know, hydrolysis was proceeding. If this time could be reduced or abolished we should be in the position of obtaining extracts which had been hydrolysed for only the reduced time or not at all.

An extract was therefore prepared so that the time of exposure of the organisms from which it was prepared—and so the time of hydrolysis—was reduced to *two* hours.



Of 33 rats inoculated with such a volume of this extract as contained 0.01 mg. nucleoprotein, 24 survived the subsequent standard infecting dose of virulent culture, = 73%. This degree of protection is less than that obtained by a similar dose of what we have called the one day's hydrolysis extract (80–90%), but taken by itself would hardly be significant.

To still further reduce the time of hydrolysis, an extract was prepared in which the use of chloroform as a killing agent was dispensed with, the bacilli being scraped off the surface of the agar

alive, washed alive, and mixed alive with the anhydrous sulphate of soda, which killed them<sup>1</sup>.

The only opportunity for hydrolysis to occur is in the final extraction of the sulphated organisms. This process occupied a short time only and took place at the temperature of the laboratory. The extract prepared from this material is therefore hydrolysed to a minimum possible under the circumstances.

Of 42 rats inoculated with a volume of this extract which contained 0.01 mg. of nucleoprotein, only 21 or 50% survived the subsequent standard injection. This experiment is distinctly significant and indicates that the initial effect of hydrolysis is to *increase* the protection afforded by the same dose of nucleoprotein, and that a well hydrolysed extract is a better vaccine than one that has been submitted to the action of the enzyme to a less degree.

If we now put in the form of a table the figures representing the protection afforded by the various extracts (percentage rats surviving the standard infecting dose) and the figures expressing the hydrolysis time, it becomes clear that so far from hydrolysis diminishing the protective value, in the early stages of the process it actually increases it.

TABLE XXIII.

*Relation between the protective value of the extracts and the amount of hydrolysis.*

Hydrolysis time	Percentage rats protected by amount of extract originally containing 0.01 nucleoprotein
Fresh	50
2 hours	73
1 day	89
1 month	83
2½ months	73

From the above experiments the very important conclusion may be drawn that *the protective and toxic properties of the extract are distinct*. These may belong either to two different substances which are difficult to separate or be two properties attached to the same substance. So far neither has been observed apart from the nucleoprotein which they accompany throughout the various manipulations to which this substance

<sup>1</sup> As long as the bacilli are manipulated alive their contents do not escape into the solution. This is however a dangerous process and to anyone who wishes to repeat it the precaution of performing the mixture in an efficient draught cupboard is advised. During the final stages of the mixing the finely powdered material floats in clouds in a slight current of air.

has been subjected. That, under the hydrolytic influence of the enzyme, the toxicity is greatly diminished whereas the protective power only manifests itself more markedly, is explicable on the assumption that both activities are proper to the nucleoprotein. If such be the case, the effect of hydrolysis suggests that the toxicity is bound up with the basic protein moiety and the immunity-conferring property with the nuclein part, as the nuclein complex is known to be highly resistant to hydrolytic agencies.

#### VI. IMMUNITY AGAINST THE TOXIN.

Hitherto when referring to immunising properties the protective effect as discovered by a subsequent inoculation of living plague *only* has been considered. Since the anti-infectious antigen appears to be independent of the toxin it was legitimate to suspect that the immunity conferred upon my rats does not depend upon the development of antitoxin. Antitoxin, however, may be produced by the repeated injection in steadily increasing amount of the toxic nucleoprotein. This aspect of the subject is treated in another report (see this *Journal*, p. 11 above) but it is necessary to mention here that by treating a horse on much the same principle as is employed in the manufacture of diphtheria antitoxin a serum of quite encouraging antitoxic value was obtained.

The following experiments were made to determine whether any resistance to the toxic extract is conferred by a previous single injection of a sublethal dose of the toxic extract or of the atoxic product resulting from the autolysis of the same.

A series of rats were injected subcutaneously with doses varying from '00005 mg. to '05 mg. of toxic nucleoprotein. The highest dose is about half that quantity which usually kills a rat in 24 hours, and one-third of all the rats which received it died before the time for determining their resistance had arrived. Hence, those ultimately tested were to some extent selected rats. The extract was similar to that which repeated experience has shown will protect against a subsequent infection by the living organisms in doses of '001 to '01 (1910, p. 559).

The tests for resistance were made one month after the inoculation. In the first series of experiments the test dose varied from '2 to 3'5 average M.L.D.'s. The results are set forth in Table XXIV and show a considerable degree of resistance to have been developed by the injection of the largest dose.

TABLE XXIV.

*Test of immunity conferred by a single dose of toxic nucleoproteid against a subsequent (one month) dose of toxic nucleoprotein.*

Test doses M.L.D.'s	Rat	Immunising dose..0.05	0.005	0.0005	0.00005 mg.
3.5	{ 1	++	+	+	+
	{ 2	0 0	0	+	+
3.0	{ 3	0 0	+	+	+
	{ 4	0 0	0	+	+
2.5	{ 5	0 0	+	+	+
	{ 6	0 0	+	0	+
2.0	{ 7	0 0	+	+	+
	{ 8	0 0	+	0	+
1.5	{ 9	0 0	0	+	+
	{ 10	0	0	+	+
1.0	{ 11	0	+	0	+
	{ 12	0	0	0	+
0.5	{ 13	0	0	0	+
	{ 14	0	0	0	0
0.2	{ 15	0	0	0	0
	{ 16	0	0	0	0

The next series of experiments was devised to ascertain whether any similar antitoxic immunity was aroused by the nucleoprotein extract after it had been mostly deprived of toxicity by hydrolysis (see p. 36 above). The toxicity of the hydrolysed material was found to be at most one-tenth of the original. It must be remembered that the atoxic extract protects equally well against infection by the living organisms.

Table XXV below, in which the resistance developed with toxic and atoxic extracts is compared, shows that the large dose .05 mg. of either confers a considerable degree of immunity, and from this experiment alone it is not possible to conclude that the toxic preparation possessed any advantage.

TABLE XXV.

*Immunity against toxin by toxic and atoxic nucleoprotein respectively.*

Immunising dose, mg...	0.05		0.005		0.0005	
	Toxic	Atoxic	Toxic	Atoxic	Toxic	Atoxic
Test dose	{	Died 2	Died 2	Died 2	Died 4	Died 7
3 M.L.D.'s		Survived 6	Survived 6	Survived 2	Survived 0	Survived 1
Test dose	{	Died 0	Died 2	Died 4	Died 3	Died 4
2 M.L.D.'s		Survived 11	Survived 5	Survived 2	Survived 5	Survived 2
Total survived		89 %	73 %	40 %	37 %	20 %
						19 %

Controls 22 %.



This result is contrary to expectation, and the matter is receiving further attention.

The conclusion that the toxicity and protective property of the extract are independent finds support from the results of experiments upon the protective value of the toxic nucleoprotein to which an anti-serum (*supra* p. 11) had been added.

A series of rats was inoculated with the serum alone, the toxic nucleoprotein alone, and with varying quantities of a neutralised mixture of the two. Altogether six series were prepared and two weeks later the degree of immunity developed by the animals was tested by the inoculation of the standard dose of the living virulent culture. The results are set forth in Table XXVI below :

TABLE XXVI.

EXP. 1. One-tenth lethal dose toxic nucleoprotein and serum to correspond.

	No. rats inoculated	No. protected	Percentage protected against subsequent infection
Serum alone ... ..	14	7	50
Toxic nucleoprotein alone	19	15	79
Mixture ... ..	15	12	80
EXP. 2. 10 neutralised lethal doses.	10	9	90
EXP. 3. 20 neutralised lethal doses.	8	8	100
EXP. 4. 50 neutralised lethal doses.	9	8	90

It will be seen from these results that the serum neutralised the toxicity without apparently affecting the substance to which protection is due. Exp. 1 in which the dose of nucleoprotein was only just optimal shows that the neutralisation of the toxicity has had no effect on the protective value of the solution. Thus the toxin may be put out of action either by neutralisation or by hydrolysis. In either case the particular antigen which is responsible for protection is, or becomes, free to act.

Working as a member of a large staff of an institute with many-sided activities, it is difficult to express individual thanks to the many of my colleagues who have helped me in the course of this investigation. I must however express my sense of obligation to Dr C. J. Martin, Director of the Lister Institute, whose constant advice and service have always been at my disposal.

## VII. CONCLUSIONS.

1. The toxic and immunising properties of the plague bacillus are attached to nucleoproteins which are soluble in water and can be extracted from the bacilli by suitable means.

2. Chloroform forms compounds with these nucleoproteins which are reversible.

3. These compounds with chloroform are relatively non-toxic and possess greatly diminished immunising powers against infection with the living organism but on removal of the chloroform the toxicity and immunising properties are regained.

4. Toluol forms similar compounds which behave in a similar manner to the analogous compounds with chloroform.

5. The effect of heat on the toxic and immunising powers of the nucleoproteins whilst in the bacillus is much less than on the toxic and immunising powers of the nucleoproteins after extraction from the bacillus. Solutions of nucleoproteins of full immunising and toxic power can be obtained from bacilli that have been heated to 60° C., whereas these properties are destroyed if solutions of the nucleoproteins exhibiting them which have been extracted from the bacilli are heated to this temperature.

6. The plague bacillus contains a proteolytic enzyme.

7. The extracts containing nucleoproteins in solution obtained under circumstances precluding as far as possible the action of this enzyme are more toxic than similar extracts obtained under circumstances allowing the enzyme to act.

8. In the early stages of the action of the enzyme on extracts containing nucleoproteins in solution exhibiting toxic and immunising powers, a fall in the value of the toxic power accompanied by a rise in the value of the immunising power occurs; by continued hydrolysis the toxicity practically disappears but the immunising power is not diminished.

9. Experiments show that some resistance against toxic extracts, as well as against infection, may be conferred by the inoculation of large doses of the same toxic extracts either in their original condition or in the atoxic modification produced by hydrolysis.

XLIV. STATISTICAL INVESTIGATION OF PLAGUE IN  
THE PUNJAB. SECOND REPORT: ON THE CON-  
NECTION BETWEEN PROXIMITY TO RAILWAYS  
AND FREQUENCY OF EPIDEMICS.

BY M. GREENWOOD, JUNR.

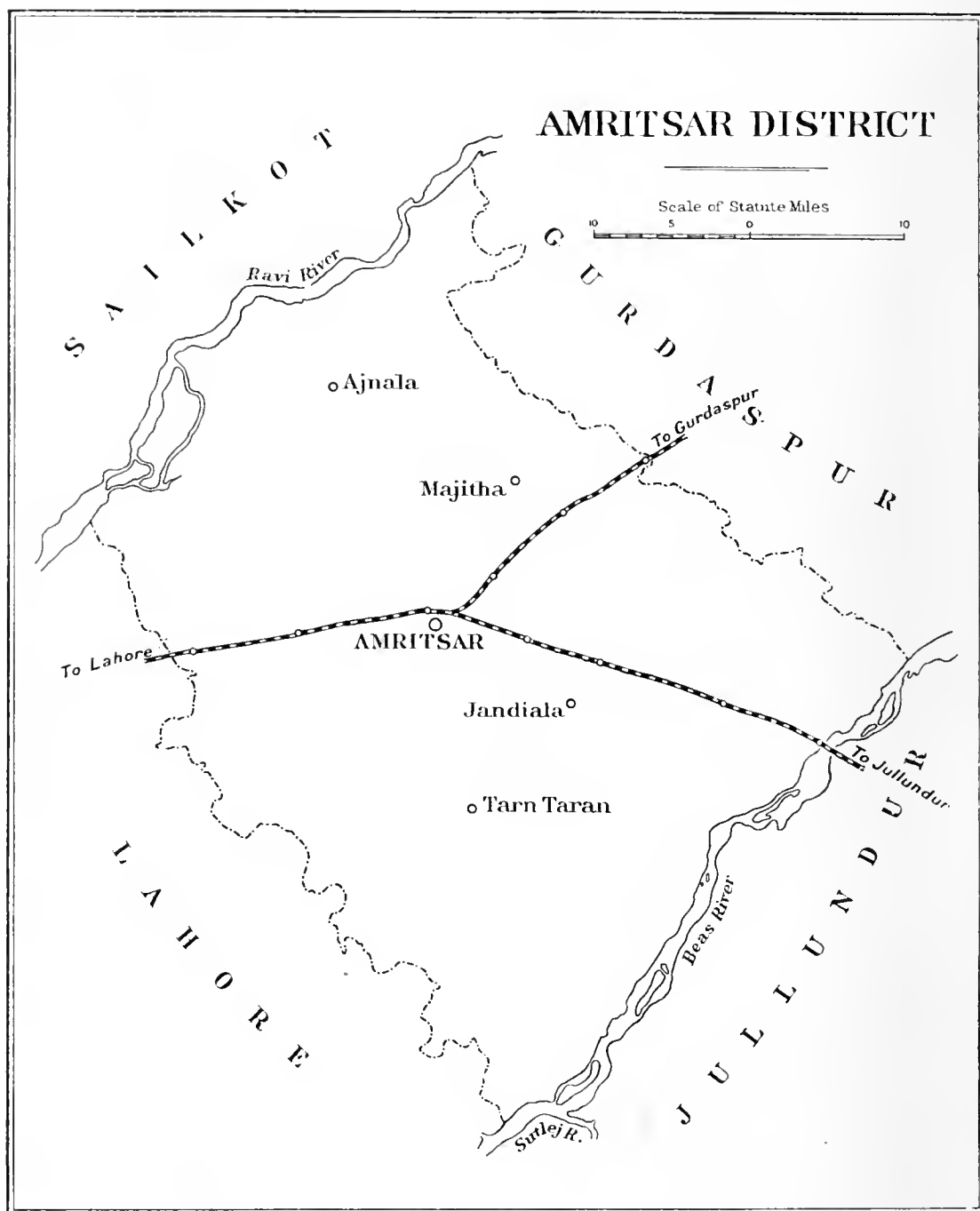
(*Statistician to the Lister Institute of Preventive Medicine.*)

With Map.

IN my former report (Vol. x. p. 424) the suggestion was made that proximity to means of communication might have an important influence on the distribution of plague epidemics within a district, and that some such disturbing factor might have been specially influential in the district of Amritsar, which is characterised by the extreme irregularity of its epidemic distribution. The present report is occupied with an analysis of material bearing on this subject.

The train of ideas which led up to the suggestion just noticed was briefly as follows. The evidence collected by Major Lamb, together with his and my analysis, tended to support the conclusion that an outbreak of plague in a village was more likely to be caused by the importation of the disease from outside, in the last resort from some relatively constant centre of infection, than by the rekindling of embers which had remained inactive since a former outbreak. But, if this view were correct, it should follow that villages in frequent communication with centres of almost constant infection, such as the large cities, ought to show a heavier rate of recurrence than villages of the same size but more secluded. The circumstances which make for free intercommunication fall into two classes. In the first place, the better a village is served by means of transit in the shape of roads and railways, the greater, other things being equal, will be the intercourse between its inhabitants and the outside world. A second class of influences cannot however be neglected. The racial peculiarities of the inhabitants, their religious and social customs and, in some cases perhaps, their occupations may make a considerable difference. It

might be that village A although badly served by railways or other means of transit is inhabited by a class of people who are peculiarly prone to migrate, while village B, in an ostensibly more favourable situa-



tion, contains a predominantly sessile population. I draw attention to these points because it is necessary to bear in mind that the complete solution of epidemiological problems such as we have to face in connection with plague demands a knowledge of many elements not at present in our

hands ; I am here concerned merely with a single one of the influences which are at work and, although the reader will perhaps concede it to be an important one, it is by no means the only nor even the prime factor. The point I have actually investigated is the connection between proximity to railways and the frequency of plague epidemics in the three tehsils of Amritsar district. I first marked on the map all villages which were situated not more than two miles from one of the three lines of railway, *i.e.* the lines from Amritsar to Lahore, Gurdaspur and Jullundur (see Map). The number so included was 155, but this figure is probably not accurate within, say, half a dozen either way, owing to the difficulty found in identifying the key numbers on the maps with the corresponding villages in the data lists, a difficulty in part due to the synonymy which is presumably an effect of transliteration.

The list obtained included two municipal towns of large size, Amritsar city and Jandiala<sup>1</sup>, which are much larger than the other villages and possibly not comparable in other respects ; in the absence of specific mention to the contrary they are not taken account of in my analysis. The remaining villages are grouped in respect of plague history as shown in Tables I and II.

The first point to notice is that the distribution is unlike that of Amritsar district taken as a whole (Table III).

Had the latter distribution been valid for the selection we should have had the state of affairs indicated in Table III B.

TABLE I.

*Summary of plague history of villages in Amritsar within two miles of the railway.*

Number of infections	Number of villages
0	13
1	16
2	27
3	37
4	32
5	24
6	4
	<hr/> 153

<sup>1</sup> Re-measurement suggests that Jandiala is somewhat more than two miles from the railway.

*Proximity to Railways*

TABLE II.

*Annual incidence of plague in the selected villages.*

Year of infection	Number of villages
Infected in 1st year	9
„ 2nd „	73
„ 3rd „	79
„ 4th „	114
„ 5th „	77
„ 6th „	97

TABLE III.

*A. General summary of the whole Amritsar district.*

Number of infections	Number of villages
0	155
1	183
2	211
3	230
4	169
5	93
6	21
	<hr/> 1062

*B. Numbers of villages within two miles of the railway actually infected compared with the expected numbers calculated on the basis of the whole district.*

Number of infections	Calculated number of villages	Actual number of villages
0	22.33	13
1	26.36	16
2	30.40	27
3	33.14	37
4	24.35	32
5	13.40	24
6	3.03	4
	<hr/> 153.01	<hr/> 153

It will noticed that the selected villages exhibit a disproportionately large number of multiple infections and an unduly small number of unaffected and once affected members. What we have to determine is whether this difference is statistically significant or capable of explanation as a mere error of random sampling. The first question that arises is as to whether the difference is an effect of size variations, since we know that villages of large size are more likely to show multiple infections

than are small ones. Tables IV and V show the size distributions of the selection (inclusive of municipal towns) and of the whole district; Table V repeats the actual figures for the selection together with the calculated figures on the supposition that the selection is a random sample in respect of size. Testing in the usual way we find  $P = \cdot 15$

TABLE IV.

*Population of villages within two miles of the railway  
(inclusive of two municipal towns).*

Population	Number of villages	Population	Number of villages
0— 300	24	3000—3300	1
300— 600	46	3300—3600	0
600— 900	25	3600—3900	1
900—1200	18	3900—4200	0
1200—1500	16·5	4200—4500	0
1500—1800	13·5	4500—4800	0
1800—2100	5	4800—5100	0
2100—2400	1	5100—5400	0
2400—2700	1	5400—up	3
2700—3000	0		<hr/> 155·0

TABLE V.

*Populations of all villages in Amritsar District.*

Population	Number of villages	Population	Number of villages
0— 300	248	3000—3300	7·5
300— 600	299	3300—3600	5
600— 900	194·5	3600—3900	4
900—1200	105·5	3900—4200	4
1200—1500	69·5	4200—4500	6
1500—1800	47·5	4500—4800	1
1800—2100	35	4800—5100	2
2100—2400	13	5100—5400	0
2400—2700	8	5400—up	6
2700—3000	6·5		<hr/> 1062·0

and, although the small groups at one end of the series tend to worsen the fit, it must be admitted that the selection contains an appreciably too small proportion of little villages, a fact which is favourable to a large number of multiple infections. In order therefore to decide whether the incidence of plague upon the villages within two miles of the railway is really excessive we must proceed further with our analysis.

Tables VII and VIII were next prepared and are self-explanatory. The reason for confining myself to villages not containing more than 2100 inhabitants was the small number and scattered distribution, in respect of size, which distinguish the larger villages. Table IX was then prepared; its method of construction will be plain from the following example.

TABLE VI.

*Expected distribution of size in the selected villages on the basis of the returns for the whole district.*

Population	Actual number	Calculated number
0— 300	24	36·20
300— 600	46	43·64
600— 900	25	28·39
900—1200	18	15·40
1200—1500	16·5	10·14
1500—1800	13·5	6·93
1800—2100	5	5·11
2100—2400	1	1·90
2400—2700	1	1·17
2700—3000	0	0·95
3000—3300	1	1·09
3300—3900	1	1·31
3900—4500	0	1·46
4500—5100	0	0·44
5100—etc.	3	0·88
	155·0	155·01

$P$  (13 groups)=0·15.

TABLE VII.

*All Amritsar villages with populations up to 2100.*

Population groups	Number of epidemics							Totals
	0	1	2	3	4	5	6	
0— 300	105	71	44	20	5	3	0	248
300— 600	43	75	81	70	23	7	0	299
600— 900	4	22	53	64	41·5	8	2	194·5
900—1200	2	10	15	29	31·5	16	2	105·5
1200—1500	0	1	11	18	25	11·5	3	69·5
1500—1800	0	1	5	7	15	18·5	1	47·5
1800—2100	1	1	2	13	7	10	1	35
Totals	155	181	211	221	148	74	9	999



TABLE VIII<sup>1</sup>.

*All selected villages with populations up to 2100.*

Population groups	Number of epidemics							Totals
	0	1	2	3	4	5	6	
0—300	9	6	6	2	0	1	0	24
300—600	4	7	13	16	4	2	0	46
600—900	0	1	4	9	9	1	0	24
900—1200	0	1	3	5	6	3	0	18
1200—1500	0	0	1	2	7	4·5	2	16·5
1500—1800	0	1	0	0	4	8·5	0	13·5
1800—2100	0	0	0	1	0	3	1	5
Totals	13	16	27	35	30	23	3	147

Let us take the case of villages thrice infected. The chance of a village between 0–300 being three times affected is (Table VII)  $\frac{20}{248}$ , and therefore in 24 villages (Table VIII) we should expect  $\frac{20 \times 24}{248}$  instances. Similarly the contribution of the villages between 300 and 600 should be  $\frac{70}{299} \times 46$ . The total number should be

$$\frac{20 \times 24}{248} + \frac{70 \times 46}{299} + \frac{64 \times 24}{194\cdot5} + \frac{29 \times 18}{105\cdot5} + \frac{18 \times 16\cdot5}{69\cdot5} + \frac{7 \times 13\cdot5}{47\cdot5} + \frac{13 \times 5}{35} = 33\cdot7.$$

Testing for goodness of fit, one finds  $P = 0\cdot10$ . The agreement is not close as it is, but the test is really too favourable to the chance of agreement for the following reasons. The totals (Table VII) upon which the prediction is based include the villages within two miles of the railway. But these latter actually form 14·7 per cent. of all the villages, hence some degree of approximation to the calculated values was only to be expected. A fairer test of differentiation seems to be to compare the actual distribution with that calculated on the basis of the villages which were more than two miles from a railway. For this purpose, Table X was prepared by subtracting the entries in Table VIII from the corresponding values in Table VII. From this table, the results given in Table XI were obtained by the process just illustrated. The value of  $P$  for this table shows that the agreement is considerably worse, that the selection exhibits too high a proportion of villages frequently infected and too low a proportion of villages rarely plague-stricken. All these results point to the conclusion that villages within reach of a railway are not a random sample of the whole of the villages. One further test may be mentioned.

<sup>1</sup> After the analysis had been completed another village was satisfactorily identified and should have figured in the selection. As no appreciable difference would be made in the results the tables have not been re-worked.

TABLE IX.

*Number of epidemics calculated for the selected villages on the basis of the whole district, attention being paid to size. For villages with populations up to 2100.*

Number of epidemics	Actual number of villages	Calculated number
0	13	17.75
1	16	23.49
2	27	30.14
3	35	33.67
4	30	25.71
5	23	14.50
6	3	1.73
	147	147.00

$P=0.10.$

TABLE X.

*Number of epidemics in villages more than two miles from the railway with populations up to 2100.*

Population groups	Number of epidemics							Totals
	0	1	2	3	4	5	6	
0— 300	96	65	38	18	5	2	0	224
300— 600	39	68	68	54	19	5	0	253
600— 900	4	21	49	55	32.5	7	2	170.5
900—1200	2	9	12	24	25.5	13	2	87.5
1200—1500	0	1	10	16	18	7	1	53
1500—1800	0	0	5	7	11	10	1	34
1800—2100	1	1	2	12	7	7	0	30
Totals	142	165	184	186	118	51	6	852

Let there be two samples  $n_1, n_2$ : let  $n_1 = p_1 + q_1$  where  $p_1$  = number marked by some character (*e.g.* the fact of having three epidemics of plague); let  $n_2 = p_2 + q_2$ . The standard deviation of  $p_1 = \sqrt{\frac{p_1 q_1}{n_1}}$  and of  $p_2 = \sqrt{\frac{p_2 q_2}{n_2}}$ . The percentage difference between the results of the two samples is  $100 \left( \frac{p_1}{n_1} - \frac{p_2}{n_2} \right)$  and the probable error of the difference is:

$$67.449 \times \sqrt{\frac{p_1 q_1}{n_1^3} + \frac{p_2 q_2}{n_2^3}} \dots\dots\dots(1).$$

TABLE XI.

*Number of epidemics calculated for the selected villages with populations up to 2100, on the basis of the rest of the district, attention being paid to size.*

Number of epidemics	Actual number of villages	Calculated number
0	13	18.52
1	16	24.61
2	27	31.23
3	35	34.19
4	30	24.95
5	23	12.10
6	3	1.40
	<hr/> 147	<hr/> 147

$$P=0.006.$$

We might compare the differences of percentages of villages infected 0, 1, 2, etc. times in each population group from the two sets of villages with the probable errors determined by (1).

This test is open to criticism. The use of the probable error based on the standard deviation as determined from the ordinary binomial requires for complete validity two assumptions, (1) that the events are really independent, (2) that the chances for and against the event are not very widely different. With regard to the latter objection, it can be met by only comparing groups in which not less than, say, 10% of the villages were infected. The former objection cannot, however, be evaded in this way. It is likely that the fact of one village being infected is not independent of the presence of infection in another village. It must, however, be observed that if plague outbreaks are mainly attributable to recrudescence and not to importation, the condition would be more nearly fulfilled, although even in that event we cannot neglect entirely the factor of common meteorological or biological conditions. On the whole, it seemed just worth while applying the test to a few groups; this has been done and the results appear in the short Table XII. Although the differences are all less than three times their probable errors, they all exceed two and a half times this function so that it may justly be said the inferences to be drawn in no way conflict with those already stated.

Having reached the conclusion that proximity to a railway does increase the liability of a village to recurrent epidemics, I attempted to measure the intensity of the relationship. To do so we must employ

the methods of multiple correlation, for we have not two variables but three, viz. (1) proximity to a railway, (2) size, (3) number of epidemics. We must determine the correlation between two of these variables for a constant value of the third, the coefficient of “partial” or “net” correlation<sup>1</sup>.

TABLE XII.  
*Comparison of certain groups of villages.*

Group		Percentage of such villages in the sample within 2 miles of the railway	Percentage of such villages among the villages more than 2 miles from the rail- way.	Difference ± probable error	Difference divided by its probable error
Population	Epidemics				
0—300	1	15·22	26·88	− 11·66 ± 4·04	2·89
300—600	3	34·78	21·34	+ 13·44 ± 5·05	2·66
600—900	4	37·50	19·06	+ 18·44 ± 6·97	2·65

I first determined the ordinary coefficients of correlation and found :

- (1) Between size of village and number of epidemics,  $r = \cdot 594$ .
- (2) Between proximity to railway and size of village,  $r = \cdot 154$ .
- (3) Between proximity to railways and number of epidemics,  $r = \cdot 265$ .

(1) was determined by the ordinary product moment method, (2) by a new process due to Pearson<sup>2</sup>, (3) from a fourfold table<sup>3</sup>, the divisions being for villages (*a*) those within two miles of the railway, (*b*) those more than two miles from the railway ; for epidemics—(*a*) not more than two epidemics inclusive, (*b*) three or more epidemics. Finally I obtained the correlation between proximity to railways and number of epidemics for a constant size of village. This was found to be  $\cdot 22$ . The question then arises as to the probable error of this coefficient. Were the partial correlation deduced from coefficients evaluated by the product moment method, it is known<sup>4</sup> that the ordinary formula can be used<sup>5</sup>. In this case, one at least of the total correlations is subject to a larger probable error than that based on product moments. I think, therefore, we should assume that the

<sup>1</sup> Yule, *Proc. Roy. Soc.* 1897, LX. 477.  
<sup>2</sup> Pearson, *Biometrika*, 1909, VII. 96.  
<sup>3</sup> Pearson, *Phil. Trans. A*, 1895, cxcv. 1.  
<sup>4</sup> Yule, *Proc. Roy. Soc. A*, 1907, LXXIX. 182. Heron, *Biometrika*, 1910, VII. 411.  
<sup>5</sup>  $0\cdot 67449 \times \frac{1-r^2}{\sqrt{n}}$ .

partial coefficient is subject to a probable error about three times the size of that given by the ordinary formula<sup>1</sup>. The formula gives in our case  $\cdot 02$ , so that we can reasonably take the correlation between proximity to railways and number of epidemics as  $\cdot 22 \pm \cdot 06$ . As the coefficient is more than three times its probable error, we may have some confidence that it indicates significant relationship between proximity to railways and number of villages.

One further point may be considered. Can we explain, on the basis of this work, the heterogeneous character of the epidemic distribution in Amritsar noticed in the first report? We can, I think, safely assert that this is one of the factors in operation, but certainly not the only one. I find on considering the villages exclusive of these within two miles of a railway, that the distribution is by no means a random one. This was to be expected, since even in regard to proximity to means of communication, the analysis here described is not exhaustive. Nearness to highways for wheeled traffic is very likely also a circumstance of importance, but, since we have evidently no satisfactory way of judging as to which roads marked in the maps are or are not greatly frequented, statistical analysis can hardly be applied to the subject.

The conclusion to be drawn from this study is therefore, that *the villages near a railway are subject to a higher rate of plague incidence than other villages and that this difference is not to be attributed to the smallness of our experience but may be considered an epidemiological fact.*

We must now inquire more particularly what is the epidemiological significance of the results obtained. Proximity to a line of railway might lead to an increased incidence of plague for all or any of the following reasons.

(1) Increased *personal* communication between villagers and centres of constant infection.

(2) Increased facility for the transit of merchandise, especially grain which might convey infected rats or possibly fleas.

(3) Some peculiarity either of the villages or the inhabitants of villages lying near the railway unconnected with (1) and (2). Since we have no extrinsic evidence pointing to condition (3), we are, for practical purposes, left to decide between (1) and (2). In a district containing large and important cities, cities of more than local fame, personal intercourse by railway is likely to be maximal, especially if any section of the

<sup>1</sup> This is a very generous allowance; in the particular case, I find, the probable error of the total coefficient (3) only 2·2 times the value deduced by the ordinary method.

inhabitants owing to racial or religious peculiarities are given to travel. On the other hand in a district without large towns personal intercommunication between villages and such towns as exist will be much less, although the railway may be extensively used for the transit of goods. Amritsar fulfils the conditions requisite for much personal intercourse. Amritsar city is one of the largest and most important, if not the most important, cities in the Punjab. The district contains a considerable percentage of Sikhs who, as I understand, are extremely prone to travel and regard Amritsar city with religious veneration. On the other hand, Rohtak is an almost purely agricultural district. It contains no towns of more than local importance and practically no Sikh inhabitants. These remarks apply in the main to a third district, Gujrat, the data from which came into my hands since the results last communicated were worked out. From the information before me, I should infer that the manufacturing or commercial interests may be stronger in Gujrat than Rohtak but that from the point of view of personal intercourse it falls into line with that district. If, therefore, (1) be the true explanation, we ought to find that the partial correlation coefficient (constant population) is larger in the case of the data for Amritsar than for Rohtak or Gujrat.

Taking the same limits of size and adopting the same measure of accuracy as for Amritsar, I calculated the various constants for Rohtak and Gujrat, finally obtaining as partial coefficients of correlation between proximity to railways and frequency of epidemics, for a constant population :

Rohtak,  $\cdot 12 \pm \cdot 09$ ,

Gujrat,  $-\cdot 18 \pm \cdot 06$ .

I have adopted the same generous margin, viz. three times the probable error given by the ordinary formula; allowing for this, we can hardly say that there is much evidence of any influence in the case of Rohtak. The coefficient for Gujrat is actually negative, pointing to some other influence, possibly a spread of infection from the margin of the district which obliterates any effect such as we have found in Amritsar.

These results amount, I think, almost to a proof of the correctness of hypothesis (1).

In our previous reports, Major Lamb and I devoted much attention to ascertaining whether the genesis of plague in the districts we investigated were in the main an affair of importation of infected

material (or animals) or due to the recrudescence of a dormant process. As stated at the beginning of this report, were the former hypothesis to express a truth, we should expect villages near an important means of communication to suffer with excessive frequency from epidemics of plague. If on the other hand, an epidemic is generally due to recrudescence, it is difficult to see why proximity to a railway should be of any importance unless we adopt (3) *supra*, which, as previously remarked, has nothing in its favour.

But not only do we conclude generally in favour of the importation hypothesis in the case of Amritsar, further we have evidence as to the conditions under which it will be valid. A comparison of the three districts seems to make it highly probable that it is importation by persons or personal effects which determines the spread of epidemics, not so much the mercantile goods' traffic.

While fully admitting the imperfections of our material from the statistical point of view, I think the conclusions just stated must be regarded as at least highly probable. It is, however, quite clear that the factor here demonstrated is not the only nor perhaps the chief one at work. This is shown by the absolutely small value of the coefficient even for Amritsar district, where the conditions are most favourable, and its total extinction in the agricultural districts of Rohtak and Gujrat.

#### CONCLUSIONS.

1. *In districts containing large cities, villages near a line of railway are liable to a higher rate of plague epidemics than villages not so situated.*
2. *This is probably due to increased opportunities for personal transit, not to the exportation or importation of merchandise.*
3. *In a district favourably situated for personal intercommunication, the spread of plague can be much better explained on the hypothesis of reimportation than on that of recrudescence.*
4. *In purely or mainly agricultural districts, proximity to railways does not increase the liability to plague outbreaks.*

## APPENDIX.

*Further note on the actual and calculated frequencies of recurrent epidemics of plague.*

In my former report (Vol. x. pp. 425-7 and Table XXIX) a special comparison was instituted between the actual frequencies of villages affected in successive years and the numbers calculated on the assumption that plague in one year did not predispose to an epidemic in the same village in the following year. In testing agreement between expectation and observation, I adopted the process used in earlier sections of the report. Now, while the value of  $\chi^2$  is evidently some measure of agreement in all cases, the value of  $P$  given by such a table as XXIX (*op. cit.*) cannot be compared strictly with the results obtained from the other tables since the whole of the chance distribution was not used. As the test presupposes a complete distribution, I have extended the comparison to the whole of the possible categories and we obtain the accompanying Table XIII. In this table the figures enclosed in brackets are the calculated frequencies on the hypothesis of independent chances. We shall obtain the same total frequency for calculated and observed values by grouping the results in several ways.

(1) If we consider each vertical column of the table as a distribution, we find the agreement to be measured by the values of  $P$  entered at the foot of each column.

(2) If we consider each sequence of two years separately and take a separate subdivision for each group of villages, giving 20 subdivisions in all, we have:

1903-4,  $P = 0.86$ ; 1904-5,  $P = 0.99$ ; 1905-6,  $P = 0.70$ ; 1906-7,  
 $P = 0.49$ .

(3) If we group all the villages together, irrespective of size, for each sequence, leaving only four groups, the values of  $P$  become 0.02, 0.78, 0.04, 0.39.

Of these arrangements (1) is perhaps to be preferred.

As will be seen from the general trend of the values, this more detailed comparison leads to precisely the same conclusion as that given in the text. In other words, the difference between observed and expected values in this series is not sufficient to warrant a belief that recurrences occur with undue frequency. The conclusions based upon this finding in the first report may therefore stand.



TABLE XIII.

*Recurrence of plague in villages: Amritsar district.*

The figures are numbers of villages, those in brackets being the numbers calculated on the supposition that the distribution of plague in one year is independent of the preceding and succeeding epidemic distribution.

Year	Population groups				
	400—500	500—700	800—1000	1000—1200	1200—1400
1903 not 1904	32 (34·35)	36 (44·73)	29 (32·07)	16 (17·97)	9 (10·35)
1904 not 1903	12 (14·35)	28 (36·73)	14 (17·07)	12 (13·97)	7 (8·35)
1903 & 1904	17 (14·65)	39 (30·27)	32 (28·93)	27 (25·03)	25 (23·65)
Neither year	36 (33·65)	63 (54·27)	22 (18·93)	12 (10·03)	5 (3·65)
1904 not 1905	14 (12·56)	22 (24·62)	4 (6·64)	6 (8·15)	5 (4·17)
1905 not 1904	40 (38·56)	60 (62·62)	41 (43·64)	20 (22·15)	13 (12·17)
1904 & 1905	15 (16·44)	45 (42·38)	42 (39·36)	33 (30·85)	27 (27·83)
Neither year	28 (29·44)	39 (36·38)	10 (7·36)	8 (5·85)	1 (1·83)
1905 not 1906	38 (39·12)	85 (87·29)	52 (55·62)	25 (28·48)	23 (25·22)
1906 not 1905	11 (12·12)	8 (10·29)	1 (4·62)	3 (6·48)	0 (2·22)
1905 & 1906	17 (15·88)	20 (17·71)	31 (27·38)	28 (24·52)	17 (14·78)
Neither year	31 (29·88)	53 (50·71)	13 (9·38)	11 (7·52)	6 (3·78)
1906 not 1907	15 (13·86)	8 (15·01)	4 (8·58)	8 (8·79)	7 (4·07)
1907 not 1906	36 (34·86)	57 (64·01)	43 (47·58)	25 (25·79)	25 (22·07)
1906 & 1907	13 (14·14)	20 (12·99)	28 (23·42)	23 (22·21)	10 (12·93)
Neither year	33 (34·14)	81 (73·99)	22 (17·42)	11 (10·21)	4 (6·93)
	$P=0·99$	$P=0·27$	$P=0·53$	$P=0·95$	$P=0·80$

# XLV. STATISTICAL INVESTIGATION OF PLAGUE IN THE PUNJAB. THIRD REPORT: ON SOME OF THE FACTORS WHICH INFLUENCE THE PREVALENCE OF PLAGUE.

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With Map and 5 Figures.

## CONTENTS.

	PAGE
I. Introduction . . . . .	62
II. Nature of the statistical materials and possible sources of error . . . . .	63
III. The relation between length of exposure to infection and total mortality . . . . .	66
IV. The relation between absolute size and relative mortality	72
V. The influence of intra-seasonal variations in meteorological phenomena upon relative mortality . . .	83
VI. On local variations in mortality . . . . .	85
VII. Summary of statistical conclusions . . . . .	89
VIII. Some general remarks upon the epidemiological problems involved in a study of village plague . . . . .	90
Appendix I. A note on English plague experience with reference to relative mortality in towns and villages . .	93
Appendix II. A note on the seasonal frequency distributions of the Punjab Districts . . . . .	96
Appendix III. Numerical factors influencing the rate of mortality	98
Appendix IV. Further note on the rates of mortality in villages of different sizes . . . . .	153

## I. *Introduction.*

IN a first report, written in conjunction with Major Lamb<sup>1</sup>, evidence was presented which made it seem probable that the recurrence of plague in the villages of the Punjab and United Provinces, which had been specially investigated, could in the main be attributed to reimportation of the disease; such a mode of origin appeared epidemiologically of greater significance than a possible awakening of

<sup>1</sup> "On the spread of epidemic plague through districts with scattered villages." *Journ. Hyg.* x. (1910), p. 350.

some *materies morbi* which had remained dormant since an earlier visitation. We also pointed out that the proportion of small villages which remained free from plague in any epidemic was much higher than that of large villages or towns (see especially Tables LIV–LIX, pp. 659–661, and I. p. 676). It was further shown that, apart from questions of size, the distribution of infected villages, particularly in the Amritsar district, suggested that certain regions might be peculiarly exposed to infection. The suggestion was made that proximity to habitual lines of travel was a factor in this result, railways being specially mentioned. In a second report (p. 47 *supra*) I have shown that, in Amritsar district, proximity to the railway has undoubtedly influenced the distribution of infected villages. At the same time, however, it was pointed out that the result of a similar inquiry in the case of another district, Gujrat, indicated that proximity to railways could not be the sole nor even the chief factor in disseminating plague.

In the two previous reports, attention was almost entirely confined to the simple fact of infection or non-infection, a division into two mutually exclusive classes being all that was attempted. Little or no heed was paid to the remarkable differences in rates of mortality among infected villages, which are nevertheless sufficiently marked in character. In order to give the reader of this report some idea of such differences, Tables I–VIII have been prepared; they are mere random samples intended as illustrations of a host of similar facts. These and kindred observations led me to formulate an epidemiological problem in the following terms—What circumstances determine the *extent* of an epidemic when plague has once manifested itself, why is it that such great discrepancies are observable in the rates of mortality? The present report is a systematic attempt to analyse the more prominent of the factors which could be thought responsible for the results. While it will undoubtedly be found that much remains obscure, I am hopeful that some light has been thrown upon points not without epidemiological importance.

## II. *Nature of the statistical materials and possible sources of error and uncertainty.*

The districts under observation are Gujrat, Hoshiapur, Rohtak and Amritsar in the Punjab, and Mozuffarnagar in the United Provinces. A few general particulars relating to these districts have been collected in Tables IX and X. It will be noticed that Amritsar is the only one

in which agriculture does not employ at least half the inhabitants and also the only one possessing a considerable urban population. The returns of plague-deaths from the numerous villages within these districts were made under the personal supervision of the late Major Lamb and numerous corrections on the data sheets together with the correspondence exchanged between Major Lamb and the local officials testify to the care with which the figures have been scrutinised. As has been frequently pointed out, however, some degree of uncertainty must always exist. Thus, when the prevalence of plague is low, there is perhaps a tendency to report deaths due to plague under other headings; conversely when the disease is widespread, deaths not caused by plague may possibly be credited to this disease. Naturally the magnitude of this error cannot be determined from internal evidence<sup>1</sup>, comparable statistics of deaths from all causes are not at hand, but its possible existence must not be ignored. A second source of error is the fact that the population returns are based on the census of 1901, *i.e.* they refer to a date several years earlier than most of the epidemics in our series. If we could be certain that all the villages have increased or decreased in the same ratio, percentages calculated on the 1901 figures while absolutely wrong would still be comparable *inter se*. Such an assumption would, however, require strong external evidence to justify it and we may be fairly confident that the (compulsory) adoption of a 1901 population basis has introduced a somewhat considerable source of error into our discussion of mortality rates. A third source of error, or rather, to speak more correctly, of uncertainty, is due to the impossibility of checking the returns of deaths in our data schedules against those which figure in the weekly lists issued by the Government of India. The summed totals from the Commission's data are widely at variance with those given in these weekly lists. It would seem, however, that the difference is not caused by any serious error in either set of figures, since there is a tolerably close agreement between both records with regard to specific towns. The most plausible suggestion is that there has been some different delimitation of the districts for the purposes of the two series. One remark may be made with regard to the weekly returns; little or no weight can be attached to case mortality deduced from a comparison of the reported seizures with the reported deaths for the same or any previous week. In many

<sup>1</sup> "From a careful study of deaths from all causes contained in the death registers we have arrived at the conclusion that very few deaths from plague escape notification." ("Plague in Belgaum," *Journ. of Hyg.* x. 1910, p. 455.)

districts such a comparison would lead (has indeed led certain newspaper writers) to the conclusion that the average case mortality is well over 90 %, an improbable figure for bubonic plague.

It will hardly be questioned that the material upon which my analysis depends is subject to very considerable errors. These errors reflect in no way upon those who have spent so much time and care upon collecting and revising the data; indeed it may safely be asserted that the present material has attained a higher standard of precision than any previous records at all commensurate with it in scope. Nevertheless, the possible sources of error are so important that statistical deductions can only be made with considerable hesitation. When the whole or most of the evidence points consistently in any one direction, we may justly have some confidence that the conclusion indicated is sound; but we must neither push our inferences too far, nor expect very detailed results. It is for these reasons that I have often presented the same material tabulated with what may seem unnecessary repetition and that I have drawn conclusions rather cautiously, even when the results of tabulation or analysis seem to force them upon one's notice. I have also in most cases omitted such subdivisions of data as would fairly involve the consideration of the so-called "errors of random sampling." I shall, indeed, from time to time refer to certain statistical questions of this nature, but in most cases such applications would only tend to clothe the results in a garment of spurious precision. In this connection I may say that tabulation has often been carried to decimal places beyond the limits of material accuracy. This has been done partly in the interests of neat statement, but more to avoid the illogical but almost instinctive conclusion on the reader's part that results given in round numbers are based on careless or approximate methods of calculation. I have thought it well to emphasize this question of "material errors" at the outset; if the subject is not again discussed in detail, I hope the reader will not imagine it to have been lost sight of and that, on the other hand, doubt or hesitation will not be attributed to pedantry. I have used my best efforts to place just as much weight upon my statistical foundations as they will bear and no more.

III. *The influence of length of exposure to infection upon total mortality.*

It has already been stated that previous work inclines one to believe that the origin of most village outbreaks is the importation from elsewhere of the disease—how this takes place will be matter for subsequent consideration. It will at once seem probable that a village which has become infected early in the season must show a greater number of cases in the whole epidemic than one not “exposed to risk” until later in the plague season, which extends in these districts roughly from November to July. Hence it might be supposed that the differences in rate of mortality as exhibited in the annual figures, which were the starting point of the present inquiry, depended merely upon this circumstance. Tables XI–XVIII contain a first study of the influence of length of exposure to infection upon total mortality; they were prepared in the following way.

All the Hoshiapur villages without exception, and all villages of less than 10,000 inhabitants in the four other districts, were grouped according to the month of first infection. That is to say, a list was made of villages which first reported cases in—*e.g.*—March, their total population, total numbers of deaths, percentage mortality, etc. were noted and the same process was applied to every other month in the plague season. The very few<sup>1</sup> villages which reported cases as early as October or November were grouped with December infections and the still fewer villages which escaped until August either omitted or reckoned in the July total. If a village had a free interval of three months or more—*e.g.* if it returned cases in January and no more until May—it was reckoned twice, once under each month. The salient features of these tables are indicated in the following notes.

*Amritsar.* 1902–3. The February infections show a higher average mortality than those of January.

1903–4. A steady decline from February. The first two months are based upon statistically inadequate returns.

1904–5. A similar picture to that of 1903.

1905–6. A practically uniform decline; there were only two infections before January.

<sup>1</sup> For the epidemics and districts where these conditions fail a separate table (XVIII A) has been prepared.

1906-7. Villages first infected in January, February or March differ little in rates of total mortality or average populations.

*Hoshiapur.* 1901-2. February and January rates similar but the mean population of the January villages markedly higher.

1903-4. February rates markedly higher than those of previous or subsequent months. (Let me repeat that what I call *e.g.* the February rate means the total rate *for the whole epidemic* of villages first infected in February, *not* the number of deaths reported in that month alone.)

1904-5. No great diminution in the rate before May, but villages first infected in December show the greatest rate.

1906-7. The marked fall does not begin until April. February worse than January.

*Gujrat.* 1903-4. Very irregular. The first three months based on too small figures.

1904-5. A fairly regular fall from the beginning but the March and April rates are sensibly equal.

1906-7. February once again predominant, but the earlier months depend on small figures.

*Mozaffarnagar.* 1903-4. Figures too small to be reliable.

1904-5. Shows a tolerably smooth fall.

1905-6. Also unreliable numbers.

1906-7. A maximum in March.

*Rohtak.* 1904-5. December below any month before April.

1906-7. February and March about equal. December and January unreliable.

The general conclusion suggested by a study of these tables would be that, on the whole, the earlier the infection is introduced the greater the resultant mortality but that the relation is neither very close nor straightforward. Let us now scrutinise the figures more closely. As will have been noticed in the tables, the average population of villages infected early in the season is generally much greater than that of villages having a longer free interval (a circumstance which might have been predicted from the evidence contained in earlier reports). It is therefore necessary to isolate the influence of earliness of infection upon total mortality from that of size; this has been attempted in two ways. Before discussing these methods, however, the reader must be clear as to exactly what problem we are now considering.

We are at present solely occupied with the influence of length of exposure upon total mortality. The plague season extends for all practical purposes when we deal with villages, not large towns, from

November to July; hence a village reporting cases in December has before it eight months during which cases might occur; if the infection does not reach the village until May then only three months can yield cases. It does not, of course, follow that these possibilities are realised, the force of the infection may spend itself in one or two months; so to speak, the danger is potential and need not become actual; this distinction will be examined later on. If now we take the number of months from the date of first infection to the end of the season as one variable and the total recorded deaths as the other variable, the correlation between the two will be a measure of the relationship between total mortality and length of possible exposure. If for number of deaths we substitute population, the correlation will measure the relation between size of village and length of exposure. It must be carefully noted that the former of these correlations does not measure the relation between any meteorological condition and the number of plague deaths. Such influences require and will receive separate consideration, but they cannot profitably be discussed until the factor of mere duration in time has been examined and measured. I now return to the methods which have been employed to study this problem.

(1) Groups of villages having nearly the same population were formed and the correlation between number of months of exposure and total deaths was determined by the direct product moment method without grouping. By this process the population factor was experimentally kept constant, but only very roughly, as will be seen.

(2) All villages of not more than 3000 inhabitants were tabulated, together with their plague histories, and the following coefficients evaluated, by the product moment method.

- (i) Absolute population and number of cases.
- (ii) Absolute population and length of possible exposure.
- (iii) Length of possible exposure and total number of deaths from plague.

We have, if  $r_{pd}$  = correlation between absolute population and length of exposure,  $r_{pm}$  = the correlation between absolute population and absolute number of deaths and  $r_{dm}$  = the correlation between absolute number of deaths and length of possible exposure,

$$\frac{r_{dm} - r_{pd} \times r_{pm}}{\sqrt{1 - r_{pd}^2} \times \sqrt{1 - r_{pm}^2}}$$

for the correlation between length of possible exposure and number of deaths for a constant population.



The results of both methods appear in Tables XIX–XXI<sup>1</sup>. Scientifically the second process is to be preferred because it was impossible to obtain a reasonably large group of villages affected in any one epidemic which were sensibly equal in populations. It is not, therefore, surprising that the coefficients in Table XIX are very variable although of the same order of magnitude as those in Table XXI. With special reference to the values of Table XXI it will be noticed that all epidemics in Hoshiapur give results in good agreement one with the other. One Gujrat coefficient is large, one Amritsar and one Mozuffarnagar value seem unduly low; in the latter case the arithmetical work has been repeated without the discovery of any slip in computation.

The question then arose whether the relatively small sizes of the coefficients were due to any marked departure from linearity so that the coefficient ceased to be a satisfactory measure of inter-relationship when taken by itself. It is hardly possible, in the present state of knowledge, to consider the partial regression coefficients directly from this point of view; sufficient information for our purpose can, however, I think, be obtained from a study of the total regression of deaths upon length of exposure. The mean numbers of deaths for each length of exposure were plotted from each set of data. The resulting points were not collinear but it was noticed that the most divergent means were based on few observations. In the case of Hoshiapur 1906–7 the nature of the regression was completely studied. In this case  $r$  was  $\cdot 281$ ,  $\eta$  (the correlation ratio<sup>2</sup>)  $\cdot 287$  and the ratio of  $\eta^2 - r^2$  to its probable error<sup>3</sup>  $1\cdot 34$ . In other words the regression was effectively linear. The diagram (Fig. 1) shows the mean points and the best straight line; it should be noted that in fitting the line each point is weighted with the number of observations on which it is based, so that the diagram exaggerates the divergence from linearity. I also found that the omission of the scattered observations derived from the early months of the epidemic did not, in the cases tested, lead to very substantial alterations in the coefficients although their values were increased. It does not, therefore, seem probable that a close relationship between length of possible exposure and total mortality has been masked by some peculiarity in the regression curve.

<sup>1</sup> Space does not permit of publishing all the correlation tables; as an illustration, the set relating to Amritsar 1902–3 is given (Tables XXI A, XXI B, XXI C).

<sup>2</sup> Pearson, *On the General Theory of Skew Correlation and Non-Linear Regression*, London, 1905.

<sup>3</sup> Blakeman, 'On Tests for Linearity of Regression in Frequency Distributions,' *Biometrika*, IV. (1905), p. 332.

I next considered the relation between possible and actual exposure, *i.e.* I ascertained whether those villages which, owing to early importation of disease, might have been infected in the greatest number of months actually were so. Table XXII shows the average durations of plague in villages first infected in each month in sequence, for two epidemics which attacked a sufficiently large number of villages to render mean values significant. Naturally the actual is less than the possible exposure but varies fairly uniformly with the latter (Fig. 2). I next attempted to measure the influence of date of first infection upon total mortality, *i.e.* I sought to discover if more cases resulted from infection *n*—*e.g.*—months 1 and 2 than from infection in months 2 and 3. In

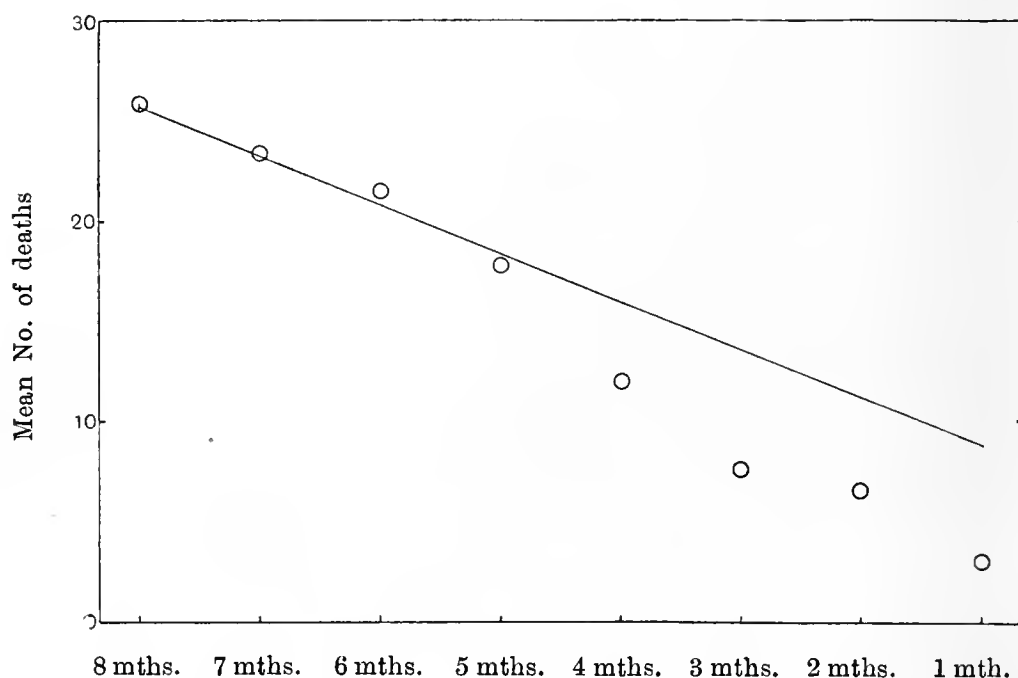


Fig. 1. Hoshiarpur, 1907. Mean no. of deaths for villages exposed during different periods. (The line is  $y = \cdot 24x$  where  $y$  is measured from the mean no. of deaths, 19.1, and  $x$  from 5.25 months. The units are 10 deaths and 1 month.)

Gujrat for the epidemic of 1906–7 (villages of populations up to 3000) villages which reported deaths in two months only were tabulated, the three coefficients of correlation were calculated and the value of the relation between date of first exposure and number of deaths for a constant population proved to be  $\cdot 26 \pm \cdot 04$ . It would therefore seem that given an equally long effective exposure there is a slight tendency for more deaths to occur early in the season than later on. All these

results tend very clearly to show that two factors influence the death rate, (a) length of possible and actual exposure, (b) *date* of first exposure. They show not less clearly that neither of these factors nor both combined suffice to explain the wide discrepancies in death rates which were the starting point of the inquiry.

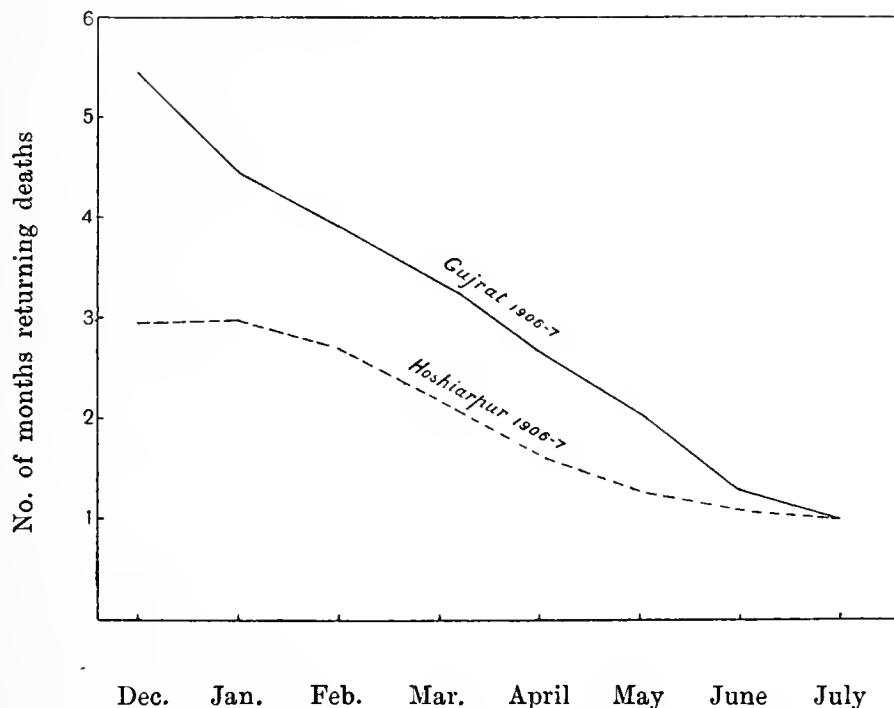


Fig. 2. Month of first infection.

The conclusions of this section may be summarised in the following way and, in view of the reasonable uniformity in analytical results, I have fair confidence that they correctly represent the epidemiological facts.

- (1) *Other things being equal*, the rate of mortality varies with the length of possible and actual exposure but the relation is not close.
- (2) Given the same period of actual exposure, more deaths will result from infections early in the epidemic season.
- (3) Large villages tend to be infected early in the season, but the relation between size and date of infection is not close.
- (4) The bulk of the difference between rates of mortality in individual villages of the same size cannot be explained completely by these results.

IV. *The relation between absolute size and relative mortality in villages.*

In Tables XXIII and XXIV, the data are so presented as to allow of a comparison between the rates of plague mortality in large and small villages. Table XXV gives the urban data while Tables XXVI—XXXII permit of a more detailed analysis of the village returns.

The general trend of these figures points to the conclusion that small villages are usually subject to a heavier rate of mortality, *when infected*, than larger ones. In certain cases, especially perhaps Gujrat, the difference is very considerable; in one instance, Rohtak, the rates go in the opposite direction. As will appear, this exception can be partly referred to the local differences which are found in Rohtak in respect of incidence, but even making this allowance, the Rohtak returns must be considered to form an exception from the present standpoint (Table XXXII)<sup>1</sup>.

Before we attempt to explain these differences it may be asked whether they can possibly be referred to errors of random sampling and a few words may be devoted to this point.

If we wish to determine the probable errors of mortality rates for plague the usual method, founded on the binomial standard deviation, is inapplicable since the condition that the events are independent one of another is not fulfilled<sup>2</sup>. The following modification of the usual process is perhaps worthy of adoption in such instances. We will take a specific example.

In Gujrat, during 1906–7, considering only villages which first reported deaths in March, the following results were obtained:

*Villages of Populations 1000—2000.*

Total population	Total deaths	Death-rate per cent.
36461	3983	10·92

*Villages of Populations less than 700.*

38522	5779	15·0
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Let us calculate the error of sampling in this case.

The weekly official returns enable us to determine the number of deaths in each month throughout the epidemic, but since the total

<sup>1</sup> See also Tables LXV and LXVI.

<sup>2</sup> See Yule, "An Introduction to the Theory of Statistics," London, 1911, Chap. XIV; Greenwood and Candy, *Journ. Roy. Statistic. Soc.* LXXIV. 1911, pp. 375–6; Yule, *ibid.* 398–9.

deaths recorded in the data prepared for Major Lamb do not correspond exactly with the weekly figures (*vide supra*) the latter were reduced to the same dimensions as the former, by multiplying throughout by

$$\frac{\text{Total Deaths in Commission's Data}}{\text{Total Deaths in the Weekly Returns}}$$

In the end, the following table was obtained :

Month	Population at risk	Chance of a plague death. Assuming independence of chances
March	234,017	·0221
April	413,096	·0247
May	505,874	·0436
June	509,820	·0199
July	503,481	·0029

We might consider each month's "exposed to risk" as a separate "population" and the chances of each person dying of plague as independent and constant *for that month*. Then, if the most probable number of deaths in any month be  $m$ , and the binomial standard deviation for the same month  $\sigma_m$ , the total number of deaths to be expected throughout the season will be  $S.m$  with a standard deviation of  $\sqrt{S\sigma_m^2}$ . The calculation was carried out as shown in the next table :

A. *Villages of Population 1000—2000.*

Month	Expected deaths	Population at risk	Square of standard deviation
March	804	36,461	786·26
April	882	35,657	860·18
May	1517	34,775	1450·82
June	662	33,258	648·83
July	96	32,596	95·72
August	—	32,500	—
3961			3841·81

S.D. 61·98. Prob. error 41·8

B. *Villages of Population 0—700.*

Month	Expected deaths	Population at risk	Square of standard deviation
March	850	38,522	831·25
April	932	37,672	908·94
May	1603	36,740	1533·07
June	699	35,137	685·09
July	101	34,438	100·70
August	—	34,337	—
4185			4059·05

S.D. 63·71. Prob. error 42·97

Finally, we reach :

Population-group	Calculated number of deaths	Actually observed number of deaths	Difference between the actual and calculated numbers divided by the latter's "probable error"
1000—2000	3961	3983	·53
0—700	4185	5779	37·1

If such a method of testing be admitted (it is at best a rough and ready process to be used with caution) the difference between the rates of mortality in the example can hardly be due to errors of random sampling.

The question of how to calculate probable errors in material of this class has a certain theoretical importance; practically, in the case before us, I do not attach any great value to the inquiry. We are only interested in large differences, and it may be doubted whether errors of random sampling are of much importance in comparison with the material errors of record mentioned above (p. 64). The numbers are large and the reader should examine the evidence as a whole, particularly Tables XXIII—XXXII. He will then, I believe, reach the conclusion that, on the average, in most epidemics the relative mortality from plague in small villages actually exposed is greater than upon large ones, the conclusion stated a few pages back. It must be remarked again that the proportion of villages which *escape altogether* is larger when we deal with the smaller communities (see Tables XXXIII, XXXIII A, and also this *Journal*, Vol. x. pp. 351, etc.). Table XXXIV illustrates the difference in the percentage rates which is effected by the inclusion not only of villages actually infected but also of those which might have been<sup>1</sup>. It will be noticed that in some, but not all, cases this inclusion has sufficed to turn the scale in the other direction. On the whole, then, a review of the somewhat considerable mass of data here collected seems to prove that, given an equal period of exposure to infection, small villages suffer more severely from plague than large ones, and we have no ground for attributing this result to errors of sampling. Can they be due to errors of record? Evidently a mistake in the returns from a small village will lead to a relatively greater error in the mortality rate than an inaccuracy of the same absolute magnitude in a village of larger size. But it would be futile to seek an explanation of our result in this way. We should have to suppose that errors of record consist in systematic over-statements of deaths in small villages and equally systematic under-statements from large villages. Such an assumption would be quite

<sup>1</sup> See also Tables LXV and LXVI.

arbitrary without positive evidence and may be dismissed. I see no reason to attribute the result to errors of record.

We must therefore attempt to interpret these curious and interesting facts but, before doing so, it is well to ask whether the same or similar circumstances have been noticed before. Hankin<sup>1</sup>, in 1905, pointed out a differential mortality rate in communities of different sizes; he contrasted village mortality with that of certain towns in Bombay. Hankin also called attention to the probable occurrence of a similar phenomenon in the Black Death. In Appendix I, I deal with such statistics as I could obtain bearing on the historic plagues; it will be found that these, so far as they go—unfortunately not very far—bear out Hankin's suggestion. They lend some support to a belief that we here have to deal with a general epidemiological phenomenon. It is to be noted that Hankin's work and my appendix are chiefly occupied with the contrast between urban and village mortality. In the case under discussion, the difference is found to be carried further and still to prevail amongst communities of the same general type.

This distinction is of epidemiological importance since the difference between a town and a village is not merely one of population, so that variation in rates of mortality might well depend upon factors which can hardly operate in village communities. In what follows I shall attend only to the latter class.

To throw light upon our problem we need first to consider how plague arises in a village and then how it spreads; let us examine these points in order.

I do not question that human epidemic plague (of bubonic type) in villages is derived from a rat epizootic by the mediation of rat fleas, particularly *X. cheopis*. Further, the evidence presented by Major Lamb and me (*op. cit.*), appeared to find its best interpretation on the hypothesis that village infections do not arise by recrudescence but from the introduction of infective material from outside. It must also be regarded as probable that neither transference by human contagion nor even by human parasites is *epidemiologically* important for the spread of village plague in the Punjab.

We shall, therefore, be justified in laying it down, as a fair working hypothesis supported by direct and indirect evidence, that in the great majority of the villages now being discussed epidemics have arisen in the following way:

<sup>1</sup> *Journ. Hyg.* v. (1905), p. 56.

At some point, or points, within the village an "effective" centre of rat plague has been established which then acts as a place of distribution for rat fleas some of which are infective. By "centre of effective occupation," I mean a place in which the rats *inhabiting* the house or hut have become infected and I distinguish an importation which has succeeded to this extent from the mere casual introduction of *materies morbi* not followed by an epizootic.

There are at least four ways in which an "effective" centre may arise:

- (1) By the introduction of an infected human being.
- (2) By the human importation, on the person or in the clothing, of infective fleas.
- (3) By the importation of infective fleas or infected rats in merchandise.
- (4) By the direct immigration of infected rats.

The last, having been specially considered by the Commission and found to have no apparent importance<sup>1</sup>, need not detain us. With regard to the other methods<sup>2</sup>, plainly, certain villages, owing to their geographical situation or some local peculiarity, might be specially prone to receive infection; but such factors have no necessary relation to size. The process initiated in the neighbourhood of an "effective" centre might be compared to a battery discharging shots—infective fleas—at random, the range being limited to a single house or group of dwellings and the rate of discharge increasing up to a certain limit. Given the establishment of one or more such primary "effective" centres, the question arises as to how the epidemic is spread through the community.

All the methods by which a centre is established *ab extra*, will still apply for the establishment of secondary centres within the community, and in addition we have to reckon with chances of spreading through the rat population by the transference of infective fleas from individual to individual. On the latter hypothesis, we should, perhaps, expect to be able to trace the lines of spread throughout the village starting from the initial centres and to find the infected areas, both epidemic and epizootic, steadily widening from week to week around these foci, until finally the distribution becomes approximately uniform.

<sup>1</sup> *Journ. Hyg. Plague* No., vii. 1907, p. 907.

<sup>2</sup> I have purposely omitted certain entomological possibilities and considerations which are of moment but not, I think, in connection with the special topic discussed in this section.



An examination of the weekly spot-maps for Belgaum<sup>1</sup> does not entirely justify such anticipations; it is impossible to trace in them any regular process of extension from initial centres throughout the community although there is evidence of a restricted area of dissemination around each focus<sup>2</sup>. The statistical evidence is equally inconclusive. Thus if plague tends to be uniformly spread through a village from the primary "effective" centres, we ought to find that the longer an outbreak endures the more nearly should the rates of mortality in different villages approximate, unless we assume that some factors correlated with absolute size arrest the extension. In other words, we ought to find that the difference between the rates of mortality in large and small villages should be greater in those only returning deaths in a single month than in villages affected in two or more months. I investigated this point, which was suggested by my colleague Dr Henderson Smith, in the way shown in Tables XXXV—XXXVIII. It cannot be said that these tables lend any real support to the idea that the difference in rates will diminish as we extend the duration of the epidemic. It cannot, of course, fairly be concluded from these results that the spread of an epidemic is not a consequence of a disseminated epizootic. The patchy character of the distribution may perhaps be accounted for in the following way. Let us suppose that at *a* and *b*, two widely separated spots, "effective" centres have been established. At and immediately around *a* and *b* there will be a considerable mortality among the rat population. This thinning of the ranks may induce an immigration of other rats so that the density of the rat population in any other places and therefore in the region between *a* and *b* will tend to be diminished. Some of the new-comers will be infected and, in course of time, the infected areas will extend, but the plague season may not last long enough for any distinct evidence of this to appear<sup>3</sup>.

In the meantime, new centres may be established at a distance from *a* and *b* by the casual emigrations of infected rats from the original points or owing to chance intercourse of the rats from *a* and *b* with individuals domiciled elsewhere.

Interesting as this problem may be, its solution is not germane to

<sup>1</sup> *Journ. Hyg.* x. (1910), pp. 446, etc.

<sup>2</sup> Cf. Brownlee, *Proc. Roy. Soc. Edinb.* xxxi. (1911), p. 262.

<sup>3</sup> It is to be remarked that the daily movements of human beings about a village tends to vitiate any attempt to trace the course of dissemination from maps showing the dwelling houses of infected persons: see this *Journal*, Vol. vii. (1907), pp. 733, 860.

the present inquiry. What *is* material, however, seems to be the apparent fact that the extent of an epidemic must largely depend upon the number of "effective" centres established early in the epidemic. The diffusive power of plague is evidently not so great that one "effective" centre is sufficient to develop a wide-spread outbreak. A sun-dried expanse of brush-wood may be completely consumed if a fire is kindled at a single point within it, but, in moister circumstances, many independent conflagrations will be required. The latter half of the simile appears to correspond to the case of plague.

On the whole we may lay it down as a working hypothesis that the extent of an epidemic will be determined in the first place by the number of independent "effective" centres established, mainly by importation, at a relatively early stage in the plague season. The degree of effectiveness of these centres will further depend on the density of the surrounding population. Now, if we return to the enumeration of possible manners in which the primary centres may be established, it seems clear *a priori*, that, as a general rule, the number of centres will increase steadily with the population. Again, if the population density be greater in large than in small villages, the efficiency of the centres should increase with the population.

An associated factor would also seem to favour an increased rate of mortality in large villages. The nearer two "effective" centres are one to another the more favourable the conditions for producing plague, since the population of the area lying between the two centres will be, if the latter are not far apart, exposed to two fires. The average distance apart of a series of balls thrown at random upon a surface of known area will depend on the number of balls and the size of the area within which they must fall<sup>1</sup>; consequently, if the area does not increase at the same rate as the number of centres, this circumstance would favour a relative increase of mortality in large villages. These *a priori* speculations lead to a result very different from the actual one. On the other hand, some considerations would point to a state of affairs qualitatively resembling what we do find.

Suppose the distribution of plague deaths *purely* random. Let the chance of a person dying from plague be  $p$  and of not dying  $q$  where

<sup>1</sup> I think, the average distance apart will vary as  $\sqrt{\frac{A}{N}}$ , where  $A$  is the number of units of area and  $N$  the number of imported foci of plague, but problems of this kind are intricate and the immediate practical importance of the matter hardly justifies a prolonged investigation.

$p + q = 1$ . Then the proportional frequency of villages, each containing  $n$  inhabitants, which will report 0, 1, 2, etc., deaths is given by the terms of the expansion of  $(p + q)^n$ . Consequently the relative frequency of non-infected villages will be greater when  $n$  is small and the rate of mortality among the infected villages higher. The subjoined figures illustrate this

No. of deaths (to nearest unit)	Binomials	
	$1000(.01 + .99)^{100}$	$1000(.01 + .99)^{300}$
0	366	49
1	370	149
2	185	224
3	61	225
4	15	169
5	3	101
6	—	50
7	—	21
8	—	8
9	—	3
10	—	1
<hr/>		
% non-infected villages ...	37.0	4.9
% deaths in infected villages	1.58	1.051

If, however, we consider any of the tables, *e.g.* Amritsar figures in Table XXXIV, it will be found that while  $p$  and  $q$  are of the order .03 and .97,  $n$  is of the order 460 and 1540 in the two groups. Thus we see that the proportion of non-infected villages in each group and the difference in rate of mortality between the groups transcend any possibility of simple explanation along these lines<sup>1</sup>. We are accordingly forced to the conclusion that the observed results require the intervention of some other factor.

Let us return to modify our conception of the way in which the spread of plague through villages can be influenced. One fairly obvious modification is with respect to the density-factor. There must, one would suppose, be an upper limit to the degree of possible overcrowding and, although we might not expect it to have been reached in villages of the size contemplated here, we may suspect that such a circumstance has operated against the uniform increase of mortality rate with population. Perhaps the density of population increases with absolute size at a steadily diminishing rate, so that we might represent the change by some expression of the form  $y = f(x)$ , where  $y$  is the

<sup>1</sup> These remarks were suggested to me by Mr G. Udny Yule to whom I am further indebted for much valuable criticism of the first draft of this chapter.

density and  $x$  the gross population, the function being such that  $y = k$  is an asymptote. But in order to account for the diminution in relative mortality as absolute size increases, it seems further necessary to suppose that (a) the number of initial centres does not increase at the same rate as the population, if we assume that the extent of an epidemic is determined by the number of such centres, or, (b), that, with an increase in population, factors limiting the epizootic extension gradually come into play. In order to decide between (a) and (b) we should have to ascertain from a large sample of villages (1) the density of population, and (2) the number of "effective" centres in the early weeks of the epizootic in each village. We have naturally no data on this scale of minuteness; some particulars can, however, be learned from the study of two Punjab villages, Dhand and Kasel.

The particulars which interest us are as follows:

Village	Population	Density per acre	Persons per house	Persons per room*	No. of houses plague-infected in 1st week of epizootic †
Dhand	1920	70	4.6	1.9	4
Kasel	3938	100	4.9	2.5	6†

\* Calculated on the assumption that houses with more than two rooms have on the average 3.5 rooms each.

† Inclusive of a house with a human case but no plague-rats.

The information is of course defective from our point of view. It cannot, for instance, be said that the presence of human cases or even plague-rats in huts early in the epizootic, constitute the houses necessarily effective centres in my sense. Again, the measure of density employed is open to criticism. Still, *prima facie*, it would seem that the change both in density and number of initial foci does not keep pace with the absolute increase of the population. It would not, of course, be difficult to construct formulae which give changes in mortality rate with increase of population comparable to the observed results. We need merely suppose that the density increases at a diminishing rate and that the number of centres increases absolutely but not relatively to the growth of population. An illustration of the sort of graphical form that can be obtained in this way is given in an appendix (Appendix III), but practically such speculations have little value for the following reasons:

(1) There are no statistics by which to test any hypothetical formula.

(2) There is no proof that the extent of an epidemic depends upon the number of primary foci of imported plague.

It is indeed possible to seek an explanation upon different lines. For instance, it has been suggested that plague introduced among rats does not spread beyond the limits of the rat colony which received the infection from without. But, if village A with  $N$  inhabitants contains two distinct colonies of rats and village B with  $\frac{N}{2}$  inhabitants only one colony, A is more likely to receive infection in one or other of its colonies than is B to suffer importation into its single colony. On the other hand, if B do get infected, the disease may be more widely disseminated through it than would be the case with a one-colony-infection in the larger village A. The principle here involved is precisely that which influenced relative mortality in favour of the small villages when we supposed the distribution of human plague to follow the simple binomial law (p. 79). I have given an arithmetical example above in dealing with that case; it will be interesting to consider the theory of the matter at a little more length in connection with the hypothesis just mentioned. It will, I believe, be found that any such interpretation is of doubtful validity.

Let  $p$  be the chance that plague will arise in a colony of rats and  $q$  the chance that it will not arise ( $p + q = 1$ ). Let us suppose that in a village with  $N$  human inhabitants there will be a single colony of rats and in a village with  $mN$  inhabitants  $m$  such colonies. Let us further assume that infection cannot be conveyed from one rat colony to another and that the number of human plague-deaths varies directly with the number of plague-stricken rat colonies so that if  $k$  human deaths are associated with one infected colony,  $mk$  deaths occur when  $m$  colonies are infected. If  $T$  villages each containing  $mN$  inhabitants and  $m$  rat colonies be exposed to risk, the average rate of mortality in the infected villages will be  $\frac{pk}{N(1-q^m)} = \frac{(1-q)k}{(1-q^m)N}$ .

The average rate in villages of  $N$  inhabitants is, of course,  $\frac{k}{N}$  and the ratio of the rates accordingly  $\frac{1-q}{1-q^m}$  which diminishes as  $m$  increases and for a constant value of  $m$  diminishes as  $q$  increases<sup>2</sup>.

<sup>1</sup> The total number of deaths

$$= Tk (mp^m + (m-1)mp^{m-1}q + \frac{(m-2)m(m-1)}{2!}p^{m-2}q^2 + \text{etc.}) = Tmpk(p+q)^{m-1} = Tmpk.$$

The population of the infected villages =  $TmN(1-q^m)$ .

$$\therefore \text{rate of mortality in infected villages} = \frac{pk}{N(1-q^m)}.$$

<sup>2</sup> Since it can be written  $\frac{1}{1+q+q^2+q^3+\dots+q^{m-1}}$ .

Now we have seen an illustration of this principle in the case of the ordinary binomial distribution obtained by assuming that human deaths from plague could be treated as simple independent occurrences. It was pointed out in this connection that the value of  $p$  deduced from the actual statistics was too large for the observed differences in mortality rates to be explicable along these lines. But the  $p$  of our present hypothesis will be larger than that of our former supposition since otherwise we should reach the absurd conclusion that the number of human plague deaths corresponding to an infected colony of rats is not less than the number of persons corresponding to a rat colony in general<sup>1</sup>.

It is further to be remarked that the fall in rate of mortality as the populations of villages exposed to risk increase is exaggerated by the hypothesis that either plague in different colonies of rats or plague in different human beings are really independent. We have supposed that when a single colony is infected there will be  $k$  human deaths and when  $m$  colonies are infected  $mk$  deaths. But it is far more likely that of two villages each containing the same population and the same number of rat colonies, say,  $m$  rat colonies, and the former having  $m$  infected colonies and the latter only one, that the mortality in the first will be more than  $m$  times that of the second. Using the notation already employed we have for the total mortality on the hypothesis of independence :

$$Tk (mp^m + m(m-1)p^{m-1}q + \dots) \dots\dots\dots(1).$$

If we imagine some cumulative effect this will be changed into an expression of, *e.g.*, the form

$$Tk\alpha (\alpha^{-\frac{1}{m}} mp^m + \alpha^{-\frac{1}{m-1}} m(m-1)p^{m-1}q + \dots) \dots\dots\dots(2),$$

where  $\alpha$  is greater than unity.

(2) will always be greater than (1) unless  $m = 1$ . Consequently the rate of mortality in infected villages of populations  $mN$  each will be greater than before.

I am accordingly unable to find a satisfactory explanation of the

<sup>1</sup> Let  $N$  = total population of a district,  $Nk_1$  the number of human deaths from plague,  $Nk_2$  the number of colonies of rats in the district,  $h$  the number of human deaths per infected colony of rats,  $p_1$  the independent chance of a colony being infected and  $p_2$  the chance of a human plague-death.  $p_1 = \frac{Nk_1}{hNk_2} = \frac{k_1}{hk_2}$  and  $p_2 = k_1$ . If  $\frac{k_1}{hk_2} < k_1$ ,  $\frac{1}{k_2h_1} < 1$ .  $\therefore k_2h > 1$ .  $\therefore h > \frac{1}{k_2}$ .  $\therefore$  no. of deaths per infected colony is greater than the average no. of persons per colony.

facts in this way. It has seemed worth discussing the more plausible hypotheses at some length because they afford an excellent illustration of the difficulty which has to be faced in attempting to give an account of a striking phenomenon<sup>1</sup>. It would appear that we require the following additional statistics before an adequate theory can be propounded. We must know :

(1) Relation between the extent of an epidemic and the number of initial centres of plague.

(2) Relation between the human and rat population of villages of different sizes.

(3) Relation between the absolute population (human) and the density of population in villages.

We also require to know whether there is any relation between absolute size and prosperity, type of house, occupations of the villagers, etc. I am aware that such information is certainly difficult and perhaps impossible to obtain for an adequate number of villages. Without it, however, all I feel justified in concluding is that a difference can generally be observed between the rates of mortality in large and small villages from the region investigated and that this difference is no mere *simulacrum* or arithmetical will-o'-the-wisp, due to the employment of ratios. I conclude the section by expressing these results in summary form :

(1) *In nearly all the districts and epidemics studied the rate of mortality in small villages which became infected was higher than in large ones.*

(2) *This phenomenon cannot be regarded as a spurious effect due to the use of relative numbers or as an error of random sampling.*

(3) *If however the populations of villages not infected are included, there is no constant difference between the rates of mortality in large and small communities.*

V. *The influence of intra-seasonal variations in rainfall and temperature upon the rate of mortality in the villages under consideration.*

It is well known that the epidemic prevalence of plague is markedly seasonal, and the Commission have already studied the particular bearing of the general facts upon the epidemiology of plague in India<sup>2</sup>. With the general conclusions arrived at in the report cited in the

<sup>1</sup> See further in Appendix IV.

<sup>2</sup> *Journ. Hygiene*, viii. 1908, p. 288 *et seq.*; also *ibid.* x. 1910, p. 446, etc.; see also Sticker, *Abhandlungen aus der Seuchengeschichte*, etc., Bd. i. Part 2, pp. 238, etc.

footnote, the present data completely agree so that it is unnecessary to consider here that aspect of the subject. One topic, however, in connection with this matter has seemed worthy of notice on the present occasion. The constancy of the general type of seasonal prevalence in any one district shows that plague will only become epidemic when the meteorological conditions (or something highly correlated with these) fall within certain limits<sup>1</sup>. It may be asked how far variations *within these limits* will alter the course and extent of the epidemic. Thus, we generally find that the mortality registered in any two epidemics is not precisely the same, often the difference is very considerable; can this be attributed to the differences in weather conditions which are found from year to year? Again, suppose that April is the month which contributes the greatest proportion of plague deaths, shall we find that the variations in the April quota from epidemic to epidemic can be brought into relation with changes in the average weather conditions of that month?

Complete data for answering these questions do not exist. In the first place we have the monthly plague figures separately recorded for only a few towns and villages; in the second, although we have numerous rainfall statistics and records of temperatures at a few places, no observations on percentage humidity are available for the districts under examination. Such data as are to be found have been summarised in Table XXXIX. Special details are given for Amritsar city in Table XL. Considering the relation of the course of several epidemics in Amritsar city and the temperature curve for Lahore, the conclusion to be drawn from a study of the facts is, I think, that both our questions must be answered in the negative. It does not seem that the relatively small changes from year to year in meteorological conditions within each part of the epidemic season can be regarded as of much importance as mortality factors. This general impression is confirmed by some applications of the method of correlation, all of which yielded negligible results. We must not hastily conclude from this that such variations are in fact without importance, but merely that their weight, in comparison with other factors, is not sufficient to reveal itself in the present kind of material. Study of the work published already by the Commission will convince the reader that the meteorological factor is of a complex nature. Its complete elucidation will require applications of the method of multiple correlation, for which the immediate statistics are not suitable.

<sup>1</sup> Some statistical points relative to the seasonal prevalence are examined in Appendix II *infra*.



VI. *On local differences in mortality.*

It will already have been noticed, in earlier tables, that the rate of plague mortality in villages infected in the same year, approximately equal in size and returning cases in the same cycle of months, exhibits not inconsiderable variations from district to district; within the same district, indeed, marked differences can be seen. This very important question will now be studied. Tables XLI–LVIII exhibit the rates of mortality in the different tehsils, or administrative subdivisions, of each district. Some notes on these tables are now given.

*Hoshiapur.* 1901–2. Una tehsil had a lower mortality than the others, but the whole outbreak was a small one.

1903–4. The same remarks apply to this year.

1904–5. Although a considerable number of villages in Una were at risk, the rate of mortality is still low.

1906–7. Una exhibits a lower rate of mortality than Garshankar or Dasuyah, but the same rate as Hoshiapur from which, paying attention to average size of village and length of exposure, it cannot be distinguished. The general experience of this district is that Una, which is separated from the rest of the district by a continuous range of hilly country, suffered less than the other tehsils. The difference is not however very striking.

*Amritsar.* 1902–3. Tarn-Tarn appears to have suffered more than either of the other tehsils, notably more than Ajnala notwithstanding the latter's lower average population.

1903–4. In this epidemic Ajnala is also relatively immune.

1904–5. Tarn-Tarn again takes the lead, the general picture being similar to that of 1902–3.

1905–6. Amritsar tehsil had many more infected villages than the other tehsils, also the heaviest rate of mortality.

1906–7. The features of 1903 and 1905 are reversed, the average population of Ajnala villages is however much less than that of the others. Evidently no general conclusion can be drawn as to the experience of this district which varied from year to year.

*Rohtak.* 1904–5. In view of its low average population, Jhajjar tehsil escaped lightly.

1906–7. The same remark applies again but very few Jhajjar villages were at risk.

Both the epidemics in Rohtak exhibit marked local divergences, a circumstance which must be taken into account when we remember

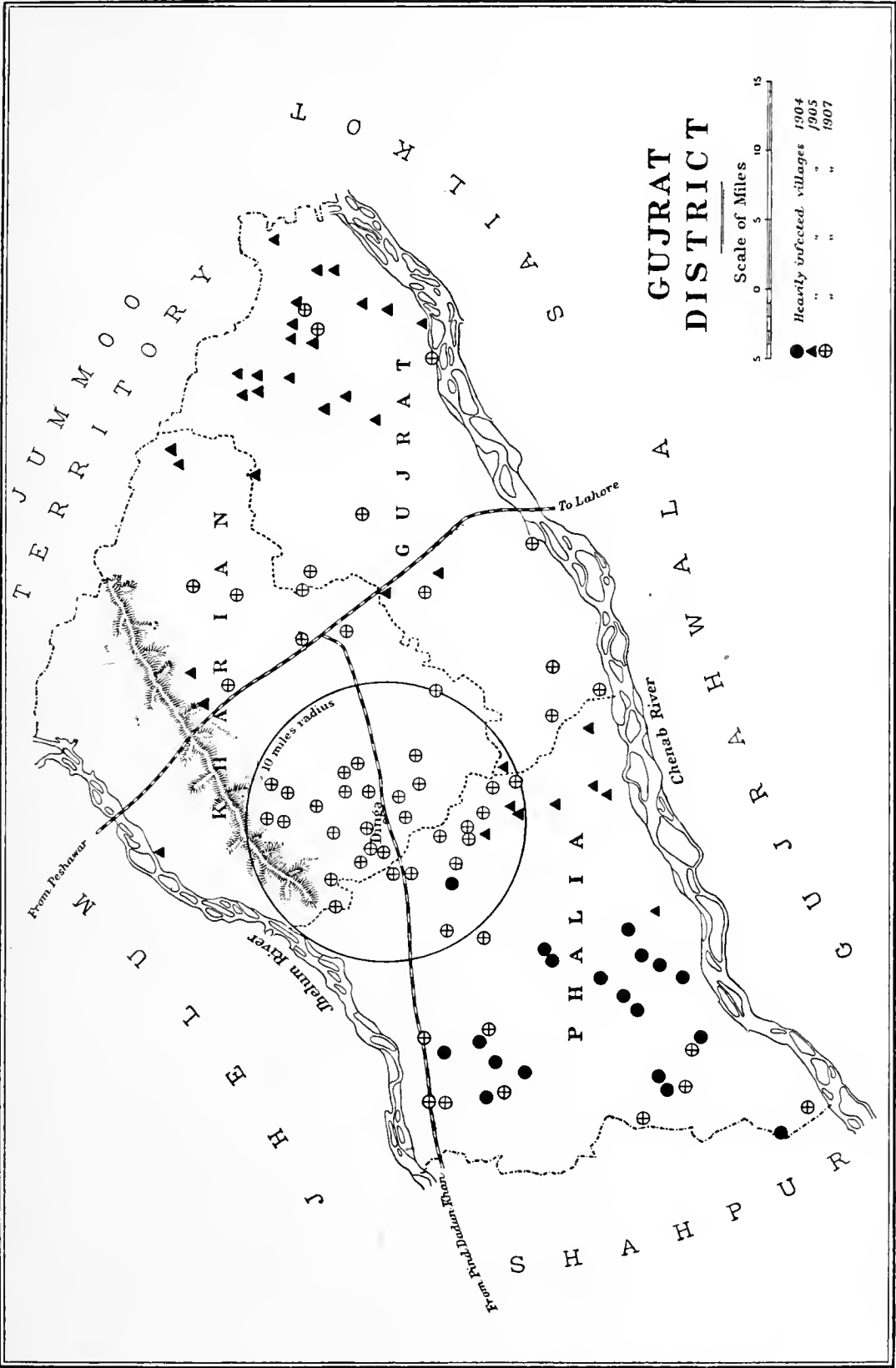
the apparent exception presented by this district to the general rule of higher mortality rates in smaller villages (*vide supra*). I do not however suggest that this is a complete explanation.

*Mozaffarnagar.* No appreciable local differences are to be found in this district.

*Gujrat.* 1903-4. Phalia tehsil exhibits an *enormously* greater mortality than the other tehsils, but also had a much larger number of villages at risk.

1904-5. No really significant differences in the local rates.

1906-7. This epidemic is the most interesting in the series because numerous villages were infected all over the district. The rate in Phalia is once again very much heavier than in Gujrat and, having regard to average population, appreciably heavier than in Kharian. Gujrat district suggested such marked local peculiarities in the incidence of plague that it was investigated in further detail. We notice in 1903-4, a decided maximum in the south-west part of the district. In 1904-5 the maximum has passed over in a north-east direction to the Gujrat tehsil. In 1905-6 less than 70 villages in all were infected, 32 in Gujrat, 12 in Kharian and 25 in Phalia, the respective rates being 1·3, 1·3 and 2·7 %. In 1906-7, the wave sweeps round to the north-west, Kharian being deeply submerged. As I have said, this year is particularly important because the average populations and total numbers of villages infected are not very different in the three tehsils. To get more exact notions, a map was prepared. Circles were marked on a map of the district to show villages which had returned deaths corresponding to a rate of three times (or more) the average mortality for the epidemic. It will be noticed that a line roughly parallel to the main railway and seven or eight miles south-west of it would leave considerably more than half the "heavily infected" villages and rather less than half the total area below and to the south-west. The most striking concentration, however, relates to the big epidemic of 1906-7 alone. The large circle on the map has its centre at Dinga railway station and a radius of ten miles. Within its circumference were 122 infected villages the populations of which could be ascertained; in the rest of the district 729 villages were infected. The average mortality within this selected area reached the enormous figure of 17·3 %, elsewhere it was less than half this. Further, only 6·6 % of the villages reported deaths as early as February while 11·5 % of the remainder were infected in this period. Some details appear in Tables LIX-LX. I think these differences are too considerable to be fairly attributed



either to errors of record or of random sampling. It would seem that some local influence was here at work which specially favoured plague ; of the nature of this factor, nothing can be gleaned from the statistics, so that it is not worth speculating here about the point, which, however, deserves attention. I think it must be admitted that some, unascertained, local peculiarities are of considerable significance, but that these factors are not necessarily constant from epidemic to epidemic. This will be seen from a general comparison of one district with another.

If we compare the districts for the three epidemics of a wide-spread character we have the following results :

District	Percentage mortalities in infected villages		
	1903—4	1904—5	1906—7
Gujrat	10·1	4·6	9·5
Rohtak	—	6·0	7·0
Amritsar	3·7	4·4	3·8
Mozaffarnagar	2·4	3·2	6·7
Hoshiapur	4·6	3·0	2·6

The differences as between the districts for the same epidemic are considerable. It will be noticed also that Gujrat and Rohtak, the districts within which we have seen the greatest local divergences in two out of the three epidemics were severely affected. Gujrat never takes a lower place than second in the order of severity. The general conclusions which seem to follow from the data here analysed can conveniently be grouped under six headings.

(1) The virulence of an epidemic in any one district varies greatly from year to year.

(2) This is partly due to some general phenomenon since the tendency is for the mortality to be high in all districts for a given epidemic or conversely.

(3) The influence is however partly local since the relative positions of the districts in respect of mortality rate are not quite the same from year to year.

(4) The importance of local factors is also demonstrated by the irregularity of incidence within any one district. An irregularity too great to be attributable to simple differences in average size, or length of exposure.

(5) The local factors, the sum of which makes in favour of a high rate of mortality, cannot be permanent in character since the subdivision

which has the heaviest mortality in one epidemic does not invariably occupy the same position in a subsequent outbreak.

(6) The rate of mortality is not necessarily heaviest in that subdivision which, judging from the number of infected villages, has plague most widely disseminated through it.

#### VII. *Summary of statistical conclusions.*

The detailed discussion of the conclusions here collected will be found in previous sections, together with the cautions necessarily to be borne in mind when weighing them (pp. 64, 65).

(1) Large villages tend to be infected earlier in the plague season than small ones.

(2) In villages of the same size total mortality is appreciably correlated with length of exposure to infection. The intensity of the relation, although quite appreciable, is not sufficient to account for the large differences in mortality rates noticed in villages of about the same size. There is some reason to think that villages infected before the months in which plague, owing to seasonal conditions, is most prevalent, do not suffer very appreciably more than villages not exposed until March or April.

(3) In nearly all cases, large *infected* villages suffer less than small ones.

(4) Plague does not occur at all in epidemic form during July, August and September in the districts analysed. The annual variations in temperature and rainfall observed during the epidemic season are not appreciably associated with variations in the rate of mortality.

(5) Differences in plague mortality in different districts, which are extremely marked, do not seem to be associated with climatic distinctions.

(6) Within any one district the variations in the rate of mortality observed in different subdivisions are considerable.

(7) A high rate of mortality in any subdivision is not necessarily accompanied by a wide dissemination of plague.

(8) There is no evidence that certain districts are *permanently* subject to a high rate of mortality. Local conditions making for an unusual degree of severity appear to vary from epidemic to epidemic.

(9) The rate of plague mortality in a village is seen to depend upon three factors, (a) length of exposure to infection, (b) number of inhabitants, (c) situation. Of these (c) is probably the most important; how it acts, however, cannot be determined from the present material.

VIII. *Some general remarks on the epidemiology of plague with special reference to village communities.*

In the following paragraphs, I shall epitomise some general considerations partly examined in the present and former reports. By adopting this course I hope to place the essential elements of the problem in a clearer light than was possible during the examination of a mass of statistical details.

The investigation of plague from the epidemiological side resolves itself into an attempt to answer three questions:

- (1) How does the disease enter a given country or district?
- (2) Having effected an entrance how does it maintain itself there?
- (3) What circumstances determine the transformation from endemic to epidemic prevalence and conversely?

(1) The majority of epidemiologists hold, I believe, that the origin of the present pandemic, the 1896 outbreak in Bombay, was probably plague ship-borne from Hong-Kong, but the point cannot be regarded as settled<sup>1</sup>.

Any identification results from the weighing of probabilities somewhat delicately balanced and complete unanimity need not be expected. If we accept the hypothesis that plague always arises from pre-existent plague, a probable but not demonstrable supposition, it is to be remarked that its implantation evidently requires the co-operation of various factors the nature of which is still obscure. It is, perhaps, generally thought that the great pandemic revivals, *e.g.* that of the sixth century, the Black Death and the present outbreak depend in the last resort upon a special accession of virulence to the specific organism. This cannot, however, be a complete explanation since, for instance, the Provence plague of 1720-1, judging from its rate of mortality, was more virulent than the worst in our Indian experience; but it did not possess wide dispersive power. In other words, virulence and diffusibility are not interchangeable expressions. This part of the epidemiological subject does not, however, specially concern the phenomena of village plagues.

(2) That the intra-local spread of human plague is due to an epizootic cannot be seriously questioned so far as India is concerned.

Nevertheless we are not warranted in asserting that this method of

<sup>1</sup> See *Report of Indian Plague Commission*, 1901, v. p. 7; *The Present Pandemic of Plague*, by J. M. Eager, Washington, 1908; E. H. Hankin, *op. cit.*; G. Sticker, *op. cit.* Part 1, p. 354; W. J. Simpson, *Treatise on Plague*, 1905, pp. 66, etc.

spread has prevailed in all epidemics elsewhere, even when the type was predominatingly bubonic. For instance, in many parts of Europe, probably in English rural districts, the Black Death was chiefly bubonic plague, but I can find no trustworthy evidence that a rat epizootic was a factor in this particular pandemic.

In the Punjab, plague is maintained as a smouldering fire in some villages during the off-season. These villages generally exceed the average size, in some cases considerably. Apart from the fact that the likelihood of infection increases with size, other considerations tend to explain this, such for instance as the greater chance of persistent infection in an absolutely large than in a small population of rats.

It must of course be clearly understood that the statistical results obtained by Major Lamb and myself do not warrant us in asserting that *all* village plagues are started by importation from a large centre; but we are justified in affirming that this method of origin has been in all probability the most usual process. Hence, our answer to (2) is briefly—plague maintains itself in towns, not to any great extent in villages. Whether centres of occupation on an endemic level in the towns themselves require re-inforcement from a permanent focus of plague, is an epidemiological problem not needing consideration at this place.

(3) Our answer to (3) in broad outline rests upon remarkably full and concordant information. We see in the Punjab, in harmony with the experience of other countries and ages, that the first prerequisite of an epidemic is the realisation of certain conditions of temperature and humidity. In the case of villages having no plague in the off-season, the probability of an epidemic happening at all depends upon ( $\alpha$ ) size; *other things being equal*, the likelihood varies inversely as size. This inter-dependence is not, of course, direct, *i.e.* a village of population  $n$  is not just twice as likely to have an epidemic as one of population  $\frac{1}{2}n$ , but increase in the percentage of infected villages as the average population rises is substantial: ( $\beta$ ) proximity to lines of transit; this being, in some but not all districts, evident when one studies the prevalence in villages near a railway. The *severity* of the epidemic in infected villages will be maximal when ( $\alpha$ ) importation occurs early in the plague season, ( $b$ ) the village is a small one. I hope to have made it seem probable that this condition depends upon some material circumstance differentiating communities of different sizes. Since, so far as I am aware, no evidence of a comparable character has been collected with the object of determining whether the same rule

holds in the case of other epidemic diseases, such as cholera or enteric fever, it cannot be stated that (b) applies to plague alone. The historical evidence *suggests* that the rule may be of general application to epidemics of plague but cannot be regarded as of probative force. (c) We must also include, as a factor regulating severity, some local condition, constant for one epidemic but variable from epidemic to epidemic, which tends to raise or lower the mortality rate in contiguous hamlets irrespective of the numbers of their inhabitants.

It would naturally be expected that a severe epidemic would tend to exhaust the susceptible persons and rats so that severe and mild outbreaks would alternate. It might also be anticipated that dispersive and toxic powers would be, *to some extent*, associated so that epidemics affecting a large number of villages might have the highest rates of mortality. These surmises are only in part justified by the statistics collected in earlier chapters of the report (*e.g.* Table X). There is no marked regularity in the succession of severe and mild epidemics and the outbreak affecting the largest number of villages has not always—although usually—shown the highest mortality rate. It would not be safe to predict that a province seriously ravaged in one year will escape lightly in the following season.

With regard to the natures of factors comprised under (c), little can be said. It seems, however, improbable that climatic changes are responsible. I hope to have proved that variations both inter- and intra-local in the mortality rate are too large to be accounted for in this way. The same remark applies to changes from year to year; the exhaustion of susceptible persons and rats plays some, possibly a considerable part; climatic changes play a less, probably much less, important *rôle*; there remains a *tertium quid* which is not apt to be placed in evidence by statistical inquiries based on existing data.

I may be permitted to hope that the analyses communicated in this report have thrown light upon some points in the epidemiology of plague in a district containing scattered villages. I recognise that, in certain directions, it has been impossible to attain results of a character sufficiently definite to warrant one in attaching much importance to them. Even in these instances, however, it is possible that the labour has not been in vain because a first step towards the attainment of definite knowledge is a summary of the imperfections in data already collected.

In conclusion I would remark that the lamented death of Major George Lamb has deprived this report of much of the value it might



otherwise have possessed. The loss of his advice and criticism in the reduction of data for the most part prepared by him has been a serious blow, and I have particularly missed that wide knowledge of local circumstances which he had at his command.

APPENDIX I.

*A note on town and village mortality rates from English plague records.*

With the exception of an outbreak in "Cadwallader's Time" and a doubtful record for 1010-11, English plague experience is confined to a period of some 350 years, commencing with the importation of the Black Death, probably at Weymouth in 1348, and ending a few years after the Great Plague of London in 1665. I have brought together in this note such scanty evidence as I have found bearing upon the relative incidence of the disease upon different population groups.

(1) The Black Death. The rate of progress of the disease can be measured in London by a comparison of wills enrolled in the Hustings Court during 1348-9 with the average for other years. Such a comparison suggests that the mortality curve reached its maximum in April-May 1349. This does not correspond to the subsequent seasonal experience of London and may perhaps be explained by a tendency towards a pneumonic type. With regard to the actual figures, Robert of Avesbury asserts that upwards of 200 burials took place daily in Manny's cemetery before Easter, not to speak of interments elsewhere. Creighton infers that the maximum daily average may have reached 200, *i.e.* about that of the worst weeks in 1563. This would give a total mortality between 20,000 and 30,000, a rate of some fifty per cent. On the basis of Seebohm's data, it would appear that village mortality surpassed this figure (*vide infra*). Some indications point to the rate having been higher in Norwich than in London. Thus, before the Black Death, the proportion of men-at-arms levied on Norwich and London were in the ratio of 6 to 10 (writ of 1351 seemingly based on a pre-1349 standard). This is a rough measure of the ratio of the populations and confirmed by the numbers of ecclesiastical parishes in the two towns (120 in London, 60 in Norwich). Thirty years later, after the Black Death and several severe outbreaks subsequently, the populations were in the ratio of 17 to 100 (estimated from the Poll Tax returns of 1377). It may be that the relative decay of Norwich is

partly attributable to the ravages of plague which seems often to have been especially severe in the Eastern counties.

Turning now to the general rate of mortality all over England (see Table LXI), two methods of inquiry have been pursued, viz. an analysis of the number of institutions in different dioceses, and a study of Manor Court Rolls. Some of the figures relating to institutions of parochial clergy, due to the researches of Jessopp and Gasquet, appear in Table A. It would seem that in some districts the mortality was greater than sixty per cent. and there is no sign of a lower rate in the sparsely populated northern districts. With respect to village mortality, Jessopp's well-known results should be mentioned. In the manor of Cornard Parva, near the boundary of Essex and Suffolk, the tenants are estimated to have been less than fifty; in six months, 21 families were obliterated; sixty deaths are noticed in the Manor Rolls, this number not including all minors and dependents.

Similar evidence is available in the cases of Hunstanton and Croxford. Jessopp concludes that "during the year ending March 1350, more than half the population of East Anglia was swept away by the Black Death. If any one should suggest that *many more* than half died, I should not be disposed to quarrel with him" (Jessopp, p. 206). Seeböhm, who investigated the rolls of the manor of Winslow, states that 153 holdings changed hands during the plague; of 43 jurors who had served in 1346-8, 27 died in 1349, a mortality of over 60 per cent. (Seeböhm, p. 29). If these results are typical, it seems clear that the village rate was not less and may have been more than that of London<sup>1</sup>.

I think the general impression to be obtained from a study of the fourteenth century pandemic is that, from the point of view of relative mortality, there is some similarity between its course and that of Indian experience; the similarity does not, however, extend to other epidemiological factors.

(2) When we come to the sixteenth and later centuries, at least one important epidemiological distinction is to be noticed. While some evidence, for example the Paston letter cited by Creighton (Creighton, p. 226), suggests that plague may have been endemic in country districts during the fifteenth century, there is no evidence of this being the

<sup>1</sup> The contemporary Simon de Corvino, in a poem on the Black Death, writes: "*Et nimis immensum sensere suburbia damnum*" (Haeser, III. p. 172). This may point to the mortality in small towns having been excessive, but the word *nimis* may have been introduced as a rhetorical expletive. I do not think we can, on the strength of this passage, cite de Corvino as an authority for the view that the Black Death was really more fatal in villages and small towns.

case in more recent times. The available facts suggest that village plagues in the sixteenth and seventeenth centuries arose by importation from towns. The question is whether the rates of mortality also contrasted. In Table LXII I have collected some of the less unreliable figures and the following records may be compared with them. In the years 1578-9, we have for London 4197 deaths (Jan. 1578-Dec. 1579), from April to December 1578, 3354 deaths. At Norwich from Aug. 20th 1578-Feb. 19th 1579, 4817 are recorded, 2335 English and 2482 aliens, giving a much heavier rate. Two thousand deaths are also reported in Yarmouth. In London during the plague year 1593, 17,844 was the total number of deaths, 10,662 from plague; in 1595 there were 3507 deaths from all causes so that the ratio of deaths in an ordinary year to deaths in a plague year would be 1:5.1. The burials in one parish of Shrewsbury during the plague year are in the ratio of 7.3:1, unity being the burials in an ordinary year. This suggests a rate certainly not below that of London. At the same period, more than 1100 deaths from plague are said to have occurred in the small city of Lichfield, but round figures of this sort are notoriously unreliable. In the town of Kirkoswald, plague appeared in 1598; the burials in that year were 583, in 1597 they numbered 42.

The famous epidemics of 1603, 1625, and 1665 tell the same story (see Table B). In the provinces, between the London plagues of 1625 and 1636, some towns experienced epidemics which would rank with the great London plagues in point of mortality rate, *e.g.* Newcastle (Creighton, p. 529).

(3) In Table LXIII I have collected data bearing upon the mortality rates of the epidemic in the South of France, 1720 (Sticker, 229). These results are indefinite but not irreconcilable with English experience.

Upon the whole, making due allowance for the fragmentary character of the material, it would appear probable that the rule deduced from the Punjab village statistics, viz. that the rate of plague mortality tends to increase as the absolute population of the infected community diminishes, may apply to European epidemics. If this be accepted, then, since it is doubtful whether the spread of the disease in the Black Death pandemic can be safely attributed to an epizootic, and it is probable that the material conditions of life in villages and small towns were inferior to those of the larger and more prosperous corporate cities, some support is afforded to the suggestion that material rather than numerical or purely epidemiological factors are responsible for the Punjab figures. It is unlikely that methods of sanitary administration, in the strict sense, can have had much to do with the results.

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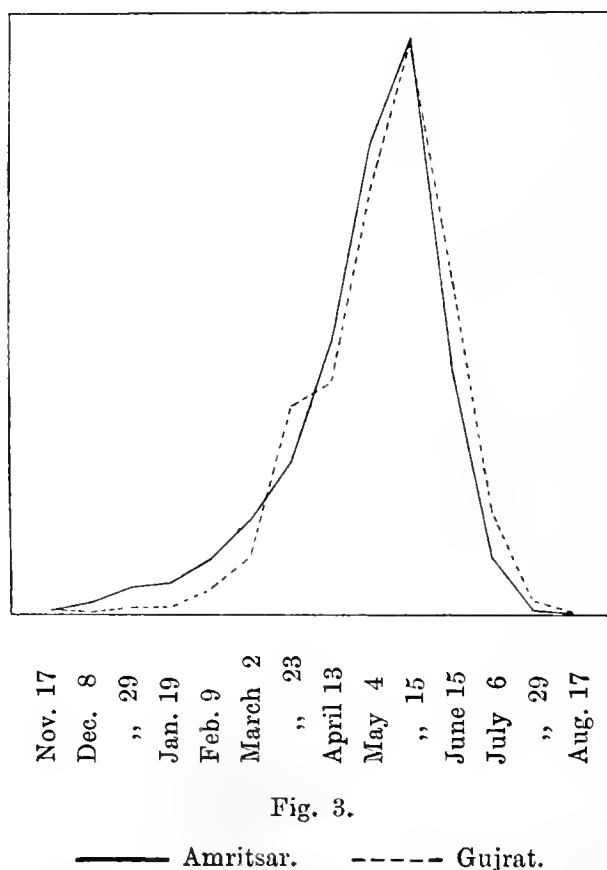
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## APPENDIX II.

*Note on the epidemic curves of Punjab districts.*

While the general form of the epidemic curve is well known and has been frequently illustrated in the Advisory Committee's reports, it appeared worth while answering two questions.

- (1) Does the form vary from one district to another?
- (2) Is there any difference in the form of a severe as compared with that of a mild epidemic?



I chose the 1906-7 epidemics for Amritsar and Gujrat districts as examples of severe outbreaks in a mainly agricultural (Gujrat) and partly urban (Amritsar) territory; 1905-6 Amritsar district illustrates the course of a relatively mild epidemic. The figures were obtained from the weekly official returns made to the Government of India. In

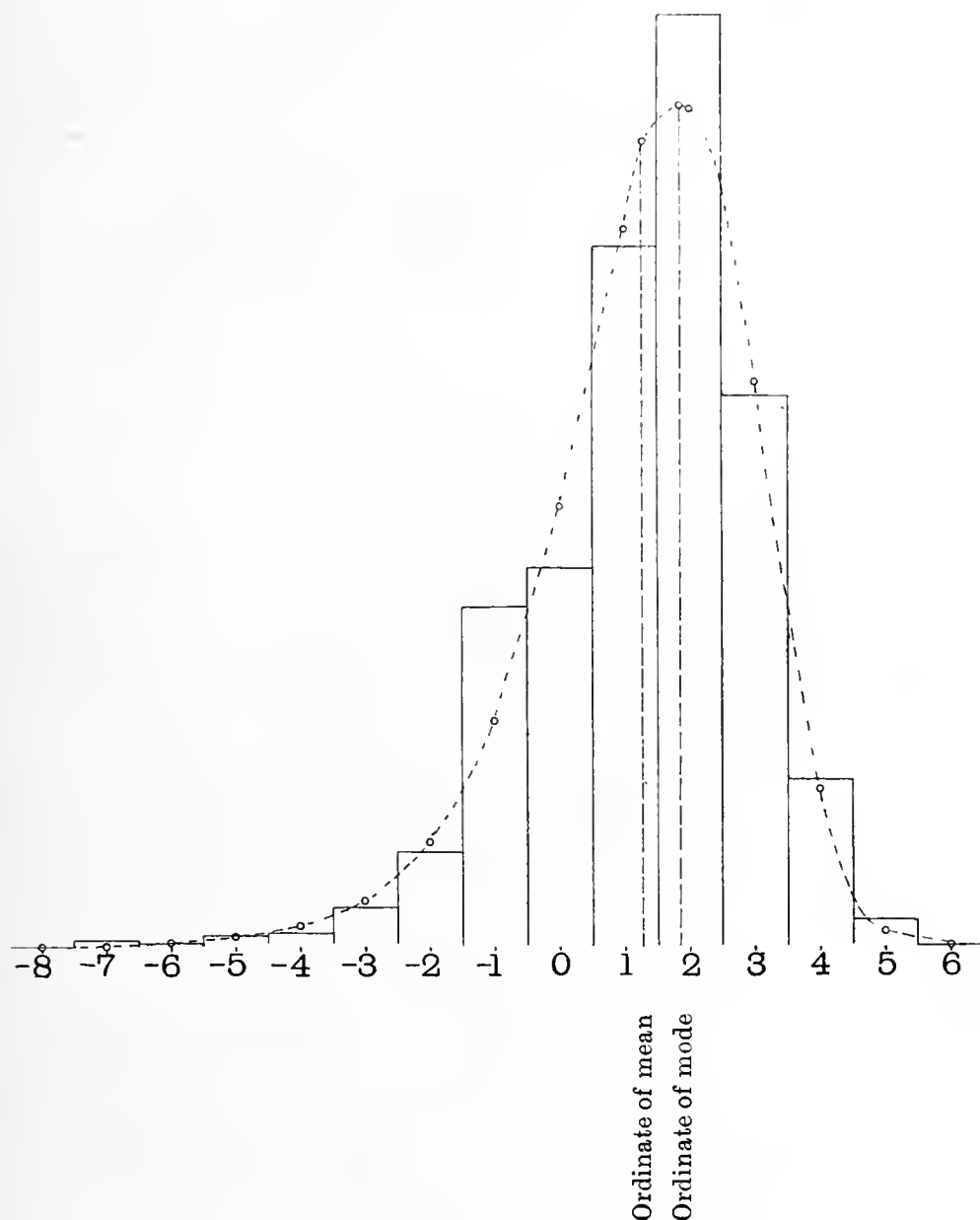


Fig. 4. Base unit, 3 weeks (the numerals refer to an arbitrary origin). Ordinate=no. of deaths from plague in each period of 3 weeks from Sept. 1906 to Sept. 1907, Gujrat. The rectangles represent the actual observations. The equation of the dotted curve is:

$$y = 1.1724 \left( 1 + \frac{x^2}{(3.8854)^2} \right)^{-8.6661} \times e^{-19.5399 \tan^{-1} \frac{x}{3.8854}}.$$

Origin at 6.2395.

the case of Amritsar, I have not entered the figures for Amritsar city when separately given.

As will be seen from the figures (Table LXIV) and diagram (Fig. 3) (in the diagram the two sets of figures are reduced to the same total) the course of the Gujrat epidemic almost exactly corresponds with that of Amritsar district. The asymmetry is marked, the fall being steeper than the rise. The minor epidemic of 1905-6 does not appear to differ in form from the wide-spread attacks.

The three sets of figures were analysed by Pearson's method. The Gujrat frequency proved to be of Type IV, Amritsar 1906-7 of Type I and Amritsar 1905-6 of Type VI. In each case the fit was poor although the Gujrat curve, which is reproduced (Fig. 4), is a tolerable representation of the course of events from the graphic standpoint. I think it is very doubtful whether the frequencies are homogeneous; there is certainly no ground for regarding them as even approximately symmetrical. In the result, so far as the data go, both questions proposed must be answered in the negative. The practical conclusion is that the factors which cut an epidemic short are more determinate than those responsible for its commencement—an inference warranted by all our other evidence.

### APPENDIX III.

#### *Note on possible interpretations of the curve of relative mortality.*

It is not difficult to frame hypotheses which would account for changes in relative mortality with size similar to those discussed in the text. The following is given as an elementary illustration; it is to be clearly understood that the assumptions made do not rest upon any reliable basis.

If the two factors which influence the rate of mortality be (1) number of initial plague centres, (2) density of population.

If, further, (1) increases more slowly than the absolute population, and if (2) increases with the population to a constant value, then

$$y = A\alpha^{\frac{k}{n+1}}\beta^{-\frac{1}{n}} \dots\dots\dots(1)$$

is a possible form, where  $y$  = the rate of mortality,  $n$  the number of inhabitants per village and  $\alpha$ ,  $\beta$ ,  $k$  and  $A$  are constants.

If, for simplicity, we put  $\alpha = \beta$  we have

$$y = A\alpha^{\frac{n(k-1)-1}{(n+1)n}} \dots\dots\dots(2).$$

Differentiating (2) we find that  $y$  has a maximum when  $n = \frac{-1 \pm \sqrt{k}}{1-k}$ .

The diagram (Fig. 5) which gives part of the curve  $y = 10^{\frac{3n-1}{(n+1)n}}$  indicates a state of affairs suggestive of the actual facts. The nature of the assumptions does not warrant us in attaching much significance to the results. The subject is, however, of interest and might be worth further investigation along these lines.

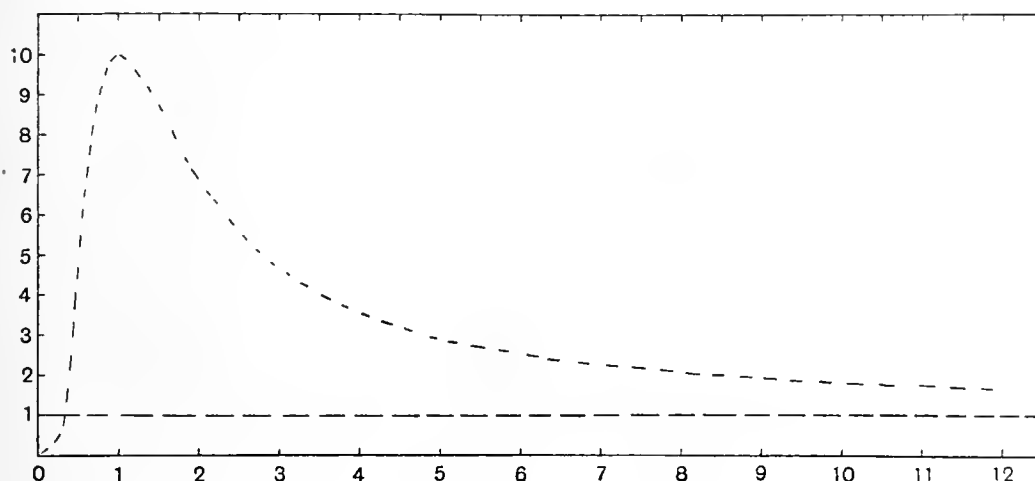


Fig. 5.

TABLE I.

РОНТАК 1905.			РОНТАК 1905.		
<i>Populations 200—250.</i>			<i>Populations 650—700.</i>		
Population	Actual number of deaths	Calculated number	Population	Actual number of deaths	Calculated number
200	20	20·29	656	1	59·79
209	75	21·21	664	130	60·52
211	5	21·41	663	60	60·43
216	6	21·92	669	9	60·98
224	21	22·73	675	67	61·52
231	4	23·44	677	173	61·71
1291	131	131·00	680	11	61·98
			681	38	62·07
			5365	489	489·00
<i>Populations 600—650.</i>			<i>Populations 700—750.</i>		
603	10	22·77	729	33	45·31
606	23	22·89	725	20	45·06
609	38	23·00	723	49	44·94
612	41	23·12	721	52	44·81
622	6	23·49	710	61	44·13
634	39	23·95	706	29	43·88
637	20	24·06	705	70	43·82
645	31	24·36	741	44	46·05
645	4	24·36	5760	358	358·00
5613	212	212·00			
<i>Populations 950—1000.</i>			<i>Populations 750—800.</i>		
951	27	77·71	751	103	39·73
954	105	77·96	759	18	40·15
956	36	78·12	766	31	40·52
957	98	78·21	782	48	41·37
964	72	78·78	796	29	42·11
974	72	79·59	796	17	42·11
981	73	80·16	4650	246	245·99
997	149	81·47			
7734	632	632·00			



TABLE II.

ROHTAK 1905.

<i>Populations 1400—1500.</i>			<i>Populations 1650—1700.</i>		
Population	Actual number of deaths	Calculated number	Population	Actual number of deaths	Calculated number
1409	92	73·05	1658	186	115·33
1415	161	73·36	1660	99	115·47
1431	176	74·19	1661	60	115·54
1432	90	74·25	1671	178	116·23
1445	12	74·92	1681	143	116·93
1467	28	76·06	1686	16	117·28
1468	85	76·11	1698	195	118·11
1474	21	76·43	1698	56	118·11
1478	10	76·63			
13,019	675	675·00	13,413	933	933·00

TABLE III.

ROHTAK 1905.

*Populations 350—400.*

Population	Actual number of deaths	Calculated number
354	4	22·32
356	33	22·45
357	18	22·51
359	97	22·64
362	7	22·83
363	3	22·89
364	21	22·95
369	7	23·27
372	37	23·46
380	5	23·96
383	19	24·15
391	31	24·66
393	6	24·78
396	30	24·97
399	35	25·16
5598	353	353·00

*Populations 250—300.*

266	5	12·92
267	2	12·97
267	11	12·97
269	16	13·07
273	1	13·26
275	8	13·36
277	13	13·46
286	25	13·89
292	26	14·19
296	18	14·38
299	24	14·53
3067	149	149·00

ROHTAK 1905.

*Populations 400—500.*

Population	Actual number of deaths	Calculated number
400	207	39·34
410	33	40·32
413	15	40·61
418	38	41·11
419	3	41·20
423	33	41·60
435	55	42·78
437	30	42·97
443	23	43·57
450	6	44·25
453	21	44·55
457	91	44·94
485	31	47·70
488	40	47·99
499	26	49·07
6630	652	652·00

*Populations 500—550.*

511	50	18·52
513	20	18·59
517	13	18·74
525	8	19·03
526	4	19·06
526	18	19·06
3118	113	113·00

TABLE IV.

RoHTAK 1905.			RoHTAK 1905.		
<i>Populations 1150—1200.</i>			<i>Populations 1000—1100.</i>		
Population	Actual number of deaths	Calculated number	Population	Actual number of deaths	Calculated number
1155	103	77·76	1004	93	52·80
1160	45	78·09	1014	96	53·32
1171	19	78·83	1031	43	54·22
1173	87	78·97	1035	38	54·43
1177	98	79·24	1042	11	54·79
1178	165	79·30	1059	6	55·69
1181	61	79·51	1059	79	55·69
1188	57	79·98	1060	3	55·74
1191	78	80·18	1064	128	55·95
1192	63	80·25	1077	115	56·63
1193	54	80·31	1084	96	57·00
1197	123	80·58	1086	14	57·11
14,156	953	953·00	1092	10	57·42
			1095	8	57·58
			1096	96	57·63
			15,898	836	836·00
<i>Populations 1200—1300.</i>			<i>Populations 1100—1150.</i>		
1298	1	77·45	1102	65	66·62
1296	76	77·33	1104	59	66·74
1297	6	77·39	1112	102	67·23
1285	39	76·68	1113	26	67·29
1285	7	76·68	1141	49	68·98
1236	275	73·75	1141	125	68·98
1228	119	73·28	1144	49	69·16
1214	82	72·44	7857	475	475·00
10,139	605	605·00			

TABLE V.

AMRITSAR 1904.			AMRITSAR 1903.		
<i>Populations 700—750.</i>			<i>Populations 700—750.</i>		
Population	Actual number of deaths	Calculated number	Population	Actual number of deaths	Calculated number
750	6	15.49	750	33	33.11
750	16	15.49	750	6	33.11
715	21	14.77	719	10	31.75
719	9	14.85	740	15	32.67
714	22	14.75	711	14	31.39
715	1	14.77	727	30	32.10
730	34	15.08	727	55	32.10
711	8	14.69	746	27	32.94
734	1	15.16	703	58	31.04
746	56	15.41	721	50	31.83
749	18	15.47	716	49	31.61
721	11	14.89	727	18	32.10
716	19	14.79	707	55	31.22
727	2	15.02	715	60	31.57
707	17	14.60	721	40	31.83
715	4	14.77	749	11	33.07
707	29	14.60	713	29	31.48
743	8	15.35	707	10	31.22
729	3	15.05	726	41	32.05
13,798	285	285.00	743	30	32.81
			14,518	641	641.00

TABLE VI.

GUJRAT 1907.			GUJRAT 1907 (cont.)		
<i>Populations 450—500.</i>					
Population	Actual number of deaths	Calculated number	Population	Actual number of deaths	Calculated number
467	1	46.79	475	2	47.59
496	73	49.70	499	21	50.00
479	37	47.99	493	70	49.40
467	34	46.79	499	19	50.00
476	27	47.69	456	20	45.69
474	4	47.49	457	188	45.79
460	28	46.09	490	23	49.10
469	2	46.99	453	18	45.39
491	29	49.20	458	54	45.89
459	2	45.99	497	170	49.80
459	2	45.99	461	68	46.19
498	6	49.90	475	41	47.59
467	17	46.79	466	60	46.69
454	35	45.49	466	75	46.69
463	32	46.39	473	33	47.39
472	42	47.29	478	114	47.89
496	134	49.70	490	22	49.10
468	100	46.89	500	21	50.10
458	18	45.89	498	45	49.90
467	174	46.79	462	64	46.29
			456	23	45.69
			19,442	1948	1948.01

TABLE VII.

GUJRAT 1907.			GUJRAT 1907 (cont.)		
Populations 750—800.			Population	Actual number of deaths	Calculated number
Population	Actual number of deaths	Calculated number			
794	11	90·81	769	77	87·95
771	7	88·18	767	270	87·73
767	98	87·73	760	70	86·93
768	103	87·84	753	66	86·12
758	5	86·70	770	43	88·07
775	145	88·64	797	31	91·16
787	62	90·01	778	67	88·98
785	135	89·79	786	89	89·90
779	220	89·10	794	16	90·81
755	295	86·35	752	60	86·01
781	62	89·33	796	66	91·04
764	27	87·38	775	94	88·64
			785	96	89·79
			19,366	2215	2214·99

TABLE VIII.

HOSHIARPUR 1902.			HOSHIARPUR 1907.		
Populations 700—750.			Populations 700—750.		
Population	Actual number of deaths	Calculated number	Population	Actual number of deaths	Calculated number
722	21	20·46	739	2	21·97
707	27	20·03	712	15	21·17
745	42	21·11	748	1	22·24
717	31	20·32	729	14	21·68
742	4	21·02	746	19	22·18
729	4	20·65	709	20	21·08
748	36	21·19	747	9	22·21
703	7	19·92	739	13	21·97
746	30	21·14	742	62	22·06
739	21	20·94	711	14	21·14
749	5	21·22	725	116	21·56
8047	228	228·00	711	1	21·14
			722	2	21·47
			707	6	21·02
			711	14	21·14
			706	34	20·99
			745	36	22·15
			734	11	21·83
			13,083	389	389·00

TABLE IX.

*General Statistical Account of the five Districts.*

RELIGIONS.

District	Total population		Hindu	Percentage of total	Sikh	Percentage of total	Mohammedan	Percentage of total	Others	Percentage of total
Amritsar	1,023,828	100·0	280,985	27·4	264,329	25·8	474,976	46·4	3538	·4
Rohtak	630,672	100·0	533,723	84·6	94	—	91,687	14·6	5168	·8
Gujrat	750,548	100·0	69,346	9·2	24,893	3·3	655,838	87·4	471	·1
Hoshiarpur	989,782	100·0	603,710	61·0	71,126	7·2	312,958	31·6	1988	·2
Mozaffarnagar	877,188	100·0	606,833	69·2	280	—	255,292	29·1	14,783	1·7

OCCUPATIONS.

District	Pasture and Agricultural	Percentage of total	Unskilled Labour (not Agricultural)	Percentage of total	Provision of Food, Drink, &c.	Percentage of total	Provision of Textile Fabrics, Furs, &c.	Percentage of total	Personal and Household Services, &c.	Percentage of total	Others	Percentage of total
Amritsar	409,690	40·0	19,005	1·9	62,242	6·1	101,467	9·9	165,611	16·2	265,813	25·9
Rohtak	385,194	61·1	7626	1·2	20,567	3·3	35,242	5·6	40,127	6·3	141,916	22·5
Gujrat	476,177	63·4	14,913	2·0	27,351	3·6	47,614	6·3	45,130	6·1	139,363	18·6
Hoshiarpur	594,180	60·0	20,898	2·1	29,296	3·0	84,354	8·5	60,974	6·2	200,080	20·2
Mozaffarnagar	449,260	51·2	98,804	11·3	50,143	5·7	53,109	6·1	86,200	9·8	139,672	15·9

TRADE.

There are no District Statistics of imports and exports and the general information as to the trade of separate districts is meagre.

Amritsar	exports wheat, shawls, carpets, cotton goods, jewellery and brass vessels, and imports (partly for re-export) grain, pulses, sugar, oil, salt, tobacco, raw cotton, cotton goods and yarn, wool, silk, shawls, blankets, metals, hardware, glass and dyes.
Rohtak	exports cereals.
Gujrat	exports wheat, millet, oil-seeds, oil, <i>ghi</i> , wool, cotton, cotton goods and hides, and imports piece goods, iron, sugar, salt, rice, wool, brass vessels, spices and dyes.
Hoshiarpur	exports rice, grain, wheat, barley, sugar, hemp and other fibres, tobacco, indigo, cotton and lac, and imports cotton and cotton yarn, piece goods, millet, cattle, dyes, salt, sugar, tobacco, kerosine, and oil-seeds.
Mozaffarnagar	exports wheat, raw sugar, barley, millet, rice and oil-seeds.

TABLE X.

*Particulars of all Epidemics in each District.*

District	Year	Total population	Number of villages infected	Total plague mortality	Death rate per cent.	Average population of infected villages
Hoshiarpur	1898	17,438	16	439	2.52	1089.9
"	1899	3791	4	9	.24	925.3
"	1900	9636	11	50	.52	876.0
"	1901	42,790	45	444	1.04	950.9
"	1902	404,430	476	11,688	2.89	849.6
"	1903	No data.				
"	1904	572,120	856	26,067	4.56	668.4
"	1905	619,241	952	19,131	3.09	650.5
"	1906	228,728	293	4125	1.80	780.6
"	1907	598,384	892	15,707	2.63	670.8
Gujrat	1902	72,200	38	551	.76	1900.0
"	1903	87,724	58	1794	2.05	1512.5
"	1904	277,151	305	23,785	8.58	908.7
"	1905	231,535	262	9782	4.22	883.7
"	1906	120,219	60	1207	1.00	2000.7
"	1907	560,579	853	51,000	9.10	657.2
Amritsar	1902	259,583	59	2039	.79	4399.7
"	1903	700,040	480	27,285	3.90	1458.4
"	1904	671,548	430	19,475	2.90	1561.7
"	1905	846,148	644	30,719	3.63	1313.9
"	1906	502,199	273	7911	1.58	1839.6
"	1907	796,099	591	24,743	3.11	1347.0
Mozaffarnagar	1902	25,581	2	4	.02	12,790.5
"	1903	73,944	23	782	1.06	3215.0
"	1904	257,455	128	5486	2.13	2011.4
"	1905	545,689	316	16,246	2.98	1726.9
"	1906	174,409	68	3077	1.76	2564.8
"	1907	753,403	590	49,909	6.62	1277.0
Rohtak	1904	147,918	56	2990	2.02	2641.4
"	1905	491,746	284	28,802	5.86	1731.5
"	1906	121,872	31	1948	1.60	3931.4
"	1907	470,442	249	31,476	6.69	1889.3

TABLE XI.

AMRITSAR.

*Epidemic of 1903.*

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '02	69,362	4324	52	62·34	1·09	1333·88
Jan.	71,890	3782	71	52·61	·92	1012·54
Feb.	84,558	5071	72	59·97	1·05	1174·42
March	104,639	5969	95	57·94	1·00	1101·46
April	89,723	4657	86	51·90	·91	1043·29
May	89,208	2533	86	28·39	·50	1037·30
June	9436	194	14	20·56	·36	674·00
	518,816	26,530	476	51·14	·883	1089·95

*Epidemic of 1904.*

Dec. '03	3879	56	2	14·44	·26	1939·5
Jan.	45,000	1715	20	38·11	·70	2250·0
Feb.	34,207	2103	23	61·48	1·12	1487·26
March	89,296	4886	70	54·72	1·00	1275·66
April	223,926	7980	207	35·64	·65	1081·77
May	92,137	1217	100	13·21	·24	921·37
June	1879	10	4	5·32	·01	469·75
	490,324	17,967	426	36·64	·670	1151·00

*Epidemic of 1905.*

Dec. '04	110,364	5826	75	52·79	1·13	1471·5
Jan.	120,386	5671	118	47·11	1·01	1020·2
Feb.	102,158	5153	95	50·44	1·08	1075·3
March	139,296	6510	128	46·74	1·00	1088·3
April	112,273	4551	125	40·54	·87	898·2
May	77,592	1729	93	22·28	·48	834·3
June	2855	38	6	13·31	·28	475·8
	664,924	29,478	640	44·33	·948	1038·94

TABLE XII.

AMRITSAR.

*Epidemic of 1906.*

Month of 1st infection	Population	No. of deaths	No. of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '05	2463	68	2	27·61	1·07	1231·5
Jan.	18,697	541	12	28·94	1·12	1558·1
Feb.	15,962	415	10	26·00	1·01	1596·2
March	64,953	1676	38	25·80	1·00	1709·3
April	97,009	2333	97	24·05	·93	1000·1
May	96,163	693	92	7·21	·28	1045·3
June	21,967	78	16	3·55	·14	1372·9
	317,214	5804	267	18·30	·709	1188·07

*Epidemic of 1907.*

Dec. '06	53,045	2701	42	50·92	1·20	1263·0
Jan.	35,877	1203	30	33·60	·79	1193·6
Feb.	60,723	2553	50	42·04	·99	1214·5
March	158,190	6732	128	42·56	1·00	1235·9
April	213,221	7528	197	35·31	·83	1082·3
May	103,421	2851	129	27·57	·65	801·7
June	17,965	260	27	14·47	·34	665·4
	642,442	23,828	603	37·09	·871	1065·4



TABLE XIII.

GUJRAT.

*Epidemic of 1904.*

Month of 1st infection	Total population	No. of deaths	No. of villages	Death rate per 1000	Mortality ratio March = 1·00	Mean population of villages
Dec. '03	13,653	912	10	66·79	·42	1365·3
Jan.	3808	1051	4	276·00	1·75	952·0
Feb.	16,529	1790	14	108·29	·69	1180·6
March	58,132	9185	68	158·00	1·00	854·9
April	98,336	9719	143	98·83	·63	687·7
May	30,568	842	46	27·55	·17	664·5
June	12,327	144	16	11·68	·07	770·4
July	620	1	1	1·61	·01	620·0
	233,973	23,644	302	101·05	·64	774·8

*Epidemic of 1905.*

Dec. '04	11,359	821	14	72·28	1·31	811·4
Jan.	8998	594	16	66·01	1·20	562·4
Feb.	8685	629	13	72·42	1·31	668·1
March	48,480	2675	52	55·18	1·00	932·3
April	70,686	3697	93	52·30	·95	760·1
May	46,857	833	61	17·78	·32	768·2
June	5027	·28	11	5·57	·10	457·0
July	—	—	—	—	—	—
	200,092	9277	260	46·36	·84	769·6

*Epidemic of 1907.*

Dec. '06	28,196	1741	16	61·75	·49	1762·3
Jan.	15,824	1909	20	120·63	·95	791·2
Feb.	40,836	5956	56	145·88	1·15	729·2
March	120,790	15,299	165	126·66	1·00	732·1
April	190,674	19,744	294	103·55	·82	648·6
May	102,997	4984	224	48·39	·38	459·8
June	26,014	563	67	21·64	·17	388·3
July	3805	39	9	10·25	·08	422·8
	529,136	50,235	851	94·94	·75	621·8

*Village Plague*

TABLE XIV.

HOSHIARPUR.

*Epidemic of 1902.*

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population of villages
Dec. '01	104,392	4543	116	43·52	3·07	899·9
Jan.	84,300	2720	89	32·27	2·28	947·2
Feb.	71,816	2342	98	32·61	2·30	732·8
March	67,473	957	79	14·18	1·00	854·1
April	47,918	896	52	18·70	1·32	921·5
May	22,864	176	34	7·70	·54	672·5
June	905	3	2	3·31	·23	452·5
July	4762	51	6	10·71	·76	793·7
	404,430	11,688	476	28·90	2·038	849·64

*Epidemic of 1904.*

Dec. '03	—	—	—	—	—	—
Jan.	62,900	2506	61	39·84	·81	1031·15
Feb.	96,191	5805	92	60·35	1·22	1045·55
March	110,282	5445	136	49·37	1·00	810·9
April	136,753	7047	249	51·53	1·04	549·1
May	127,600	4595	243	36·01	·73	525·1
June	30,176	446	62	14·78	·30	486·7
July	8218	223	13	27·14	·55	632·15
	572,120	26,067	856	45·56	·923	668·36

TABLE XV.

HOSHIARPUR.

*Epidemic of 1905.*

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population of villages
Dec. '04	71,162	3534	98	49·66	1·59	726·14
Jan.	194,770	6495	230	33·35	1·07	846·83
Feb.	87,999	2391	119	27·17	·87	739·49
March	82,962	2585	144	31·16	1·00	576·13
April	96,801	2640	186	27·27	·88	520·44
May	49,109	977	96	19·89	·64	511·55
June	29,774	423	65	14·21	·46	458·06
July	6664	86	14	12·91	·41	476·00
	619,241	19,131	952	30·89	·991	650·46

*Epidemic of 1907.*

Dec. '06	73,603	2475	99	33·63	1·14	743·46
Jan.	119,808	3346	123	27·93	·95	974·05
Feb.	96,984	3069	139	31·64	1·06	697·73
March	133,924	3937	224	29·40	1·00	597·88
April	113,387	2248	192	19·83	·67	590·56
May	39,691	569	90	14·34	·49	441·01
June	18,463	108	20	5·85	·20	923·15
July	2748	15	5	5·46	·19	549·60
	598,608	15,767	892	26·35	·893	671·10

TABLE XVI.  
MOZAFFARNAGAR.

*Epidemic of 1904.*

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '03	19,419	651	6	33·52	1·25	3236·5
Jan.	18,315	616	7	33·63	1·25	2616·4
Feb.	38,581	1331	20	34·50	1·28	1929·1
March	58,233	1567	30	26·91	1·00	1949·1
April	57,720	822	46	14·24	·53	1254·8
May	10,954	138	9	12·60	·47	1217·1
June	7663	24	7	3·13	·12	1094·7
July	—	—	—	—	—	—
	210,885	5149	125	24·42	·908	1687·08

*Epidemic of 1905.*

Dec. '04	137,988	4975	62	36·05	1·17	2225·6
Jan.	82,538	3012	48	36·49	1·18	1719·5
Feb.	39,781	1402	29	35·24	1·14	1371·8
March	126,554	3908	82	30·88	1·00	1543·34
April	67,114	2029	54	30·23	·98	1242·85
May	34,343	546	34	15·90	·51	1010·1
June	3069	7	4	2·28	·07	767·3
July	—	—	—	—	—	—
	491,387	15,879	313	32·31	1·046	1569·93

TABLE XVII.  
MOZAFFARNAGAR.

*Epidemic of 1906.*

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population per village
Dec. '05	24,829	473	9	19·05	·66	2758·8
Jan.	3428	214	2	62·43	2·15	1714·0
Feb.	17,309	1014	8	58·58	2·02	2163·6
March	28,423	825	18	29·03	1·00	1579·1
April	27,346	298	19	10·90	·38	1439·3
May	15,895	85	8	5·35	·18	1986·9
June	2699	1	1	·37	·013	2699·0
July	—	—	—	—	—	—
	119,929	2910	65	24·26	·836	1845·06

*Epidemic of 1907.*

Dec. '06	83,580	3746	41	44·82	·503	2038·5
Jan.	87,134	7504	50	86·12	·95	1742·7
Feb.	133,234	10,522	81	78·97	·88	1644·9
March	145,205	13,126	133	90·40	1·00	1091·8
April	160,761	9665	174	60·12	·67	923·9
May	80,158	1952	99	24·35	·27	809·7
June	9022	122	10	13·52	·15	902·2
July	—	—	—	—	—	—
	699,094	46,637	588	66·77	·739	1188·94

*Village Plague*

TABLE XVIII.

ROHTAK.

*Epidemic of 1905.*

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population of villages
Dec. '04	63,620	3500	25	55·01	·64	2544·8
Jan.	97,979	7387	41	75·39	·88	2389·7
Feb.	67,554	4588	43	67·92	·79	1571·0
March	82,796	7080	57	85·51	1·00	1452·6
April	114,258	4687	95	41·02	·48	1202·7
May	30,624	401	19	13·09	·15	1611·8
June	1095	8	1	7·31	·09	1095·0
Total	457,926	27,651	281	60·38	·71	1629·6

*Epidemic of 1907.*

Dec. '06	38,129	3603	11	94·50	1·16	3466·3
Jan.	29,508	3141	11	106·40	1·58	2228·0
Feb.	57,078	4400	26	77·09	·96	2195·3
March	169,885	13,799	85	81·23	1·00	1998·7
April	96,351	5220	73	54·18	·67	1319·9
May	42,900	643	35	14·99	·18	1225·7
June	4290	43	6	10·02	·12	715·0
Total	438,141	30,849	247	70·40	·88	1753·6

TABLE XVIII A.

*Particulars of villages infected in December or earlier and grouped together in Tables XI—XVIII.*

HOSHIARPUR 1907.

Month of 1st infection	Population	Deaths	No. of villages	Mortality %	Average population of villages
July	7010	283	7	4·04	905·8
August	434	20	3	4·61	144·7
September	764	3	2	·39	382·0
October	4803	142	8	2·96	600·4
November	28,136	832	35	2·96	803·9
December	32,456	1195	44	3·68	737·6
Total	73,603	2475	99	3·36	743·5

AMRITSAR 1907.

September	1409	72	2	5·11	704·5
October	3469	121	3	3·49	1156·3
November	17,175	1039	14	6·05	1226·8
December	30,992	1469	23	4·74	1347·5
Total	53,045	2701	42	5·09	1263·0

MOZAFFARNAGAR 1907.

October	12,266	515	7	4·20	1752·3
November	21,436	799	8	3·73	2679·5
December	49,878	2432	26	4·88	1918·4
Total	83,580	3746	41	4·48	2036·6

TABLE XIX.

*Relation between length of exposure and number of deaths in the whole epidemic.*

Population group	Number of villages	Number of deaths	Mean deaths per village	Population	Mean population of villages	Standard deviation of population	Coefficient of variation	<i>r</i>
Hoshiarpur 1902 :								
10—200	46	380	8.3	5416	117.7	56.5	47.98	+ .44 ± .08
130—270	57	576	10.1	11,763	206.4	40.2	19.47	+ .41 ± .07
250—350	57	565	9.9	17,067	299.4	28.6	9.56	+ .37 ± .08
350—450	56	683	12.2	22,099	394.6	29.0	7.36	+ .40 ± .08
450—550	41	797	19.4	20,365	496.7	28.5	5.73	+ .29 ± .10
525—675	58	987	17.0	34,641	597.3	42.4	7.10	+ .31 ± .08
625—775	41	905	22.1	28,626	698.2	50.2	7.19	+ .47 ± .08
700—900	51	1318	25.8	41,009	804.1	55.7	6.92	+ .46 ± .07
800—1000	48	1544	32.2	43,073	897.4	61.3	6.83	+ .29 ± .09
875—1125	50	1676	33.5	49,710	994.2	70.5	7.09	+ .38 ± .08
Hoshiarpur 1905 :								
75—125	48	357	7.4	4765	99.3	16.0	16.07	- .06 ± .10
125—175	62	481	7.8	9228	148.8	15.0	10.09	+ .41 ± .07
175—225	73	671	9.2	14,818	203.0	14.5	7.14	+ .36 ± .07
225—275	75	776	10.3	18,642	248.6	15.7	6.32	+ .21 ± .07
275—325	65	494	7.6	19,438	299.0	14.7	4.91	+ .14 ± .08
325—375	76	1135	14.9	26,799	352.6	15.0	4.24	+ .34 ± .07
375—425	50	636	12.7	20,001	400.0	15.2	3.79	+ .18 ± .09
425—475	61	837	13.7	27,186	445.7	15.1	3.40	+ .48 ± .07
475—525	41	639	15.6	20,529	500.7	14.8	2.95	+ .43 ± .09
520—580	45	750	16.7	24,846	552.1	18.7	3.39	+ .26 ± .09
550—650	75	1369	18.3	44,972	599.6	29.0	4.84	+ .29 ± .07
650—750	41	680	16.6	28,747	701.1	28.5	4.06	+ .33 ± .09
750—850	47	1138	24.2	37,658	801.2	30.4	3.79	+ .38 ± .08
840—960	40	970	24.3	35,957	898.9	35.6	3.96	+ .40 ± .09
925—1075	48	1374	28.6	47,846	996.8	46.1	4.63	+ .38 ± .08
1000—1200	42	1215	28.9	45,617	1086.1	53.9	4.97	+ .17 ± .10
1050—1350	49	1771	36.1	57,901	1181.7	92.3	7.81	+ .35 ± .08
1150—1450	42	1739	41.4	54,940	1308.1	80.3	6.14	+ .28 ± .10
1200—1600	50	2489	49.8	69,018	1380.4	99.9	7.24	+ .24 ± .09
1300—1700	45	2316	51.5	65,412	1453.6	107.6	7.40	+ .11 ± .10
1400—1800	38	1997	52.6	60,092	1581.4	127.5	8.06	+ .28 ± .10
Hoshiarpur 1907 :								
75—125	50	664	13.3	4984	99.7	14.1	14.12	+ .18 ± .09
175—225	62	681	11.0	12,395	199.9	15.9	7.93	+ .37 ± .07
275—325	51	638	12.5	15,178	297.6	14.5	4.87	+ .14 ± .09
370—430	60	655	10.9	24,135	402.3	19.9	4.94	+ .28 ± .07
450—550	70	1046	14.9	34,631	494.7	28.8	5.82	+ .27 ± .07
550—650	67	1229	18.3	40,143	603.2	31.2	5.17	+ .60 ± .05
650—750	40	720	18.0	27,839	695.4	31.8	4.58	+ .12 ± .11
725—875	64	1203	18.8	51,111	798.6	42.3	5.30	+ .25 ± .08
825—975	57	1234	21.6	51,243	899.0	47.4	5.27	+ .48 ± .07
925—1075	46	1100	23.9	46,029	1000.6	47.3	4.73	+ .34 ± .09
990—1210	42	1243	29.6	45,282	1078.1	56.3	5.23	+ .20 ± .10
1025—1375	55	1440	26.2	64,837	1178.9	117.7	9.98	+ .37 ± .08
1125—1475	43	1212	28.2	56,538	1314.8	96.7	7.36	+ .50 ± .08
1200—1600	48	1358	28.3	67,108	1398.1	97.4	6.96	+ .40 ± .08
1300—1700	49	1588	32.4	71,416	1457.5	107.6	7.38	+ .47 ± .08
1400—1800	40	1285	32.1	63,344	1583.6	122.1	7.71	+ .59 ± .07
Amritsar 1903 :								
100—300	31	845	27.3	6864	221.4	50.1	22.63	+ .09 ± .12
400—500	45	1333	29.6	20,528	456.2	28.2	6.18	- .02 ± .10

TABLE XIX (continued).

Population group	Number of villages	Number of deaths	Mean deaths per village	Population	Mean population of villages	Standard deviation of population	Coefficient of variation	r
Amritsar 1903 (continued):								
700— 900	72	2849	39.6	57,254	795.2	53.5	6.70	+ .13 ± .08
900—1100	48	2792	58.2	47,709	993.9	64.9	6.53	+ .00 ± .10
1050—1350	56	3254	58.1	66,495	1187.4	90.0	7.58	+ .35 ± .08
1150—1450	47	3182	67.7	60,434	1285.8	85.6	6.66	+ .21 ± .09
1250—1550	48	3354	69.9	67,270	1401.5	85.2	6.08	+ .20 ± .09
1350—1650	43	2916	67.8	64,428	1498.3	78.7	5.25	+ .22 ± .10
1450—1750	43	3094	72.0	67,986	1581.1	91.9	5.81	+ .45 ± .08
1700—2300	45	3776	83.9	86,493	1922.1	146.5	7.62	+ .33 ± .09
1850—3150	43	4133	96.1	96,943	2254.5	374.1	16.59	+ .35 ± .09
2000—4000	40	4750	118.8	108,251	2706.3	572.7	21.16	+ .49 ± .08
Amritsar 1904:								
100— 300	42	610	14.5	9536	227.0	48.7	21.44	+ .41 ± .09
400— 500	25	477	19.1	11,240	449.6	28.3	6.28	- .38 ± .12
700— 900	56	1259	22.5	44,196	789.2	54.0	6.84	+ .11 ± .09
900—1100	41	2051	50.0	40,785	994.8	56.5	5.68	+ .48 ± .08
1050—1350	50	2522	50.4	59,898	1198.0	89.1	7.44	+ .58 ± .06
1150—1450	42	2181	51.9	53,703	1278.6	81.5	6.38	+ .46 ± .08
1250—1550	43	2467	57.4	59,972	1394.7	89.7	6.43	+ .35 ± .09
1350—1650	40	2849	71.2	60,091	1502.3	84.9	5.65	+ .39 ± .09
1450—1750	44	2743	62.3	69,952	1589.8	89.9	5.65	+ .25 ± .10
1700—2300	42	2853	67.9	81,198	1933.3	155.4	8.04	+ .39 ± .09
1900—3100	39	2543	65.2	90,782	2327.7	334.8	14.38	+ .13 ± .11
2000—4000	40	2901	72.5	105,171	2629.3	530.2	20.17	+ .10 ± .11
Amritsar 1905:								
100— 300	60	1290	21.5	13,668	227.8	46.9	20.57	+ .20 ± .08
400— 500	55	1329	25.2	25,005	454.6	27.9	6.15	+ .42 ± .07
700— 900	91	3591	39.5	72,668	798.5	54.4	6.81	+ .33 ± .06
900—1100	60	3301	55.0	59,318	988.6	64.5	6.52	+ .35 ± .08
1100—1300	45	2313	51.4	53,814	1195.9	58.3	4.87	+ .53 ± .07
1200—1400	41	2040	49.8	53,165	1296.7	60.4	4.66	+ .18 ± .10
1250—1550	58	3345	57.7	81,257	1401.0	83.5	5.96	+ .17 ± .09
1350—1650	57	3410	59.8	84,968	1490.7	85.1	5.71	+ .18 ± .09
1450—1750	52	2961	56.9	80,789	1553.6	74.8	4.82	+ .34 ± .08
1700—2300	51	3438	67.4	98,925	1939.7	158.2	8.16	+ .13 ± .09
1900—3100	46	4020	87.4	106,765	2321.0	329.7	14.21	+ .27 ± .09
2000—4000	53	4904	92.5	138,576	2614.6	549.3	21.01	+ .16 ± .09
Amritsar 1907:								
100— 300	60	1266	21.1	13,284	221.4	52.5	23.73	+ .31 ± .08
400— 500	52	1258	24.2	23,393	449.9	26.9	5.98	+ .30 ± .09
700— 900	82	3000	36.6	65,685	801.0	55.1	6.87	+ .29 ± .07
900—1100	57	2136	37.5	56,481	990.9	64.2	6.48	+ .33 ± .08
1100—1300	45	1825	40.6	53,932	1198.5	56.9	4.75	+ .28 ± .09
1150—1450	55	2408	43.8	70,383	1328.0	82.2	6.19	+ .25 ± .09
1250—1550	49	2405	49.1	68,259	1393.0	86.0	6.17	+ .41 ± .08
1350—1650	44	2284	51.9	65,672	1492.5	83.4	5.59	+ .42 ± .08
1450—1750	44	2086	47.4	69,781	1585.9	86.8	5.47	+ .41 ± .08
1700—2300	53	3106	58.6	102,469	1933.4	147.9	7.65	+ .26 ± .09
1900—3100	47	3594	76.5	108,123	2300.5	329.0	14.30	+ .19 ± .09
2000—4000	50	4081	81.6	131,778	2635.6	539.3	20.46	+ .24 ± .09
Gujrat 1907:								
400— 500	83	3914	47.2	37,385	450.4	28.0	6.22	+ .35 ± .06
750— 850	41	3432	83.7	32,650	796.3	30.7	3.86	+ .21 ± .10
900—1100	52	4777	91.9	52,338	1006.5	62.2	6.18	+ .29 ± .09
1050—1350	47	4945	105.2	54,167	1152.5	70.5	6.12	+ .15 ± .10

TABLE XX.

*Mean values of coefficients in Table XIX.*

District	Year	Mean $r$	S.D.	Coeff. of var.
Amritsar	1903	·23	·154	66·96
	1904	·33	·221	66·97
	1905	·27	·113	41·85
	1907	·30	·066	22·00
Hoshiarpur	1902	·38	·062	16·32
	1905	·29	·122	42·07
	1907	·35	·144	41·14
Gujrat	1907	·27	·079	29·26

TABLE XXI.

*Correlation between length of exposure and total number of plague deaths for a constant population.*

(Villages up to 3000 inhabitants.)

District	Epidemic	No. of villages	Coeff. of correlation
Hoshiarpur	1901—2	447	·25 ± ·03
„	1903—4	844	·24 ± ·02
„	1904—5	950	·24 ± ·02
„	1905—6	284	·29 ± ·04
„	1906—7	875	·25 ± ·02
Gujrat	1903—4	296	·39 ± ·03
„	1904—5	257	·27 ± ·04
„	1906—7	843	·28 ± ·02
Rohtak	1904—5	232	·30 ± ·04
„	1906—7	200	·30 ± ·04
Amritsar	1902—3	456	·19 ± ·03
„	1903—4	412	·34 ± ·03
„	1904—5	643	·29 ± ·02
„	1906—7	560	·28 ± ·03
Mozaffarnagar	1904—5	266	·05 ± ·04
„	1906—7	536	·27 ± ·03

*Mean values for different districts.*

Hoshiarpur	·25	Amritsar	·28
Gujrat	·30	Mozaffarnagar	·20
Rohtak	·30		

TABLE XXI A.

*Amritsar (1902-3). Month of first infection.*

Population group	December	January	February	March	April	May	June	Frequency
0—200	1.0	1.0	2.0	3.0	4.0	4.0	—	15.0
200—400	3.0	10.0	5.0	2.0	12.0	14.0	5.0	51.0
400—600	4.0	13.0	14.0	23.0	8.0	17.0	2.0	81.0
600—800	4.0	12.0	12.0	16.0	16.0	12.0	4.0	76.0
800—1000	10.0	6.0	8.0	14.0	12.0	8.0	1.0	59.0
1000—1200	7.0	6.0	9.0	6.0	8.0	3.0	1.0	40.0
1200—1400	7.0	8.0	2.5	7.0	3.0	7.0	—	34.5
1400—1600	1.0	1.0	2.5	6.0	10.0	10.0	—	30.5
1600—1800	4.0	5.0	3.0	6.0	3.0	3.0	—	24.0
1800—2000	6.0	4.0	5.0	1.0	2.0	2.0	—	20.0
2000—2200	—	1.0	2.0	4.0	1.0	4.0	1.0	13.0
2200—2400	1.0	—	—	1.0	2.0	—	—	4.0
2400—2600	—	1.0	—	1.0	1.0	1.0	—	4.0
2600—2800	—	—	—	—	1.0	—	—	1.0
2800—3000	—	1.0	1.0	1.0	—	—	—	3.0
Total	48.0	69.0	66.0	91.0	83.0	85.0	14.0	456.0

TABLE XXI B.

*Amritsar (1902-3). Population groups.*

Number of deaths	0—200	200—400	400—600	600—800	800—1000	1000—1200	1200—1400	1400—1600	1600—1800	1800—2000	2000—2200	2200—2400	2400—2600	2600—2800	2800—3000	Frequency
1—5	3.0	2.0	4.0	2.0	3.0	2.0	2.0	1.0	—	—	1.0	—	—	—	—	20.0
6—15	2.0	15.0	16.5	14.0	8.0	5.5	2.0	3.0	—	1.5	1.0	—	—	—	—	68.5
15—30	8.0	18.0	25.5	19.0	10.0	5.5	5.0	5.5	4.0	3.0	2.0	—	—	—	—	105.5
30—45	1.0	5.0	18.0	15.0	8.5	3.5	7.5	4.0	2.5	1.5	1.0	—	1.0	—	—	68.5
45—60	1.0	3.0	7.5	15.5	12.0	6.5	5.0	6.0	6.5	.5	1.5	1.0	.5	—	—	66.5
60—75	—	3.5	4.5	4.5	5.5	6.5	3.0	—	—	4.5	.5	—	.5	—	—	33.0
75—90	—	.5	1.5	1.0	5.5	5.0	1.5	2.0	1.5	2.5	1.0	1.0	—	—	—	23.0
90—105	—	1.5	2.5	4.0	4.0	2.0	3.5	5.0	3.5	3.5	4.0	1.0	—	—	—	34.5
105—120	—	.5	—	—	1.0	1.5	—	1.0	2.0	—	—	—	—	—	—	6.0
120—135	—	—	—	1.0	.5	—	—	—	—	—	1.0	—	—	—	2.0	4.5
135—150	—	1.0	1.0	—	—	—	.5	1.0	—	—	—	—	.5	—	1.0	5.0
150—165	—	1.0	—	—	—	—	.5	—	—	—	—	—	.5	1.0	—	3.0
165—180	—	—	—	—	—	.5	.5	—	1.0	—	—	—	1.0	—	—	3.0
180—195	—	—	—	—	—	.5	.5	1.0	1.5	—	—	.5	—	—	—	4.0
195—210	—	—	—	—	—	1.0	1.0	—	.5	—	—	.5	—	—	—	3.0
210—225	—	—	—	—	1.0	—	—	—	—	1.0	—	—	—	—	—	2.0
225—240	—	—	—	—	—	—	1.0	—	—	—	—	—	—	—	—	1.0
240—255	—	—	—	—	—	—	.5	1.0	1.0	—	—	—	—	—	—	2.5
255—270	—	—	—	—	—	—	.5	—	—	1.0	—	—	—	—	—	1.5
270—285	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—	1.0
Total	15.0	51.0	81.0	76.0	59.0	40.0	34.5	30.5	24.0	20.0	13.0	4.0	4.0	1.0	3.0	456.0



TABLE XXI c.

*Amritsar (1902-3). Month of first infection.*

Number of deaths	December	January	February	March	April	May	June	Frequency
1— 5	4.0	5.0	—	2.0	2.0	6.0	1.0	20.0
6— 15	5.5	13.0	8.5	9.0	6.0	18.0	8.5	68.5
15— 30	12.0	17.0	5.5	16.5	22.0	28.0	3.5	104.5
30— 45	3.5	7.0	13.5	15.5	10.5	18.5	1.0	69.5
45— 60	4.0	6.5	11.5	15.5	18.5	10.5	—	66.5
60— 75	2.0	7.5	6.0	8.0	8.5	—	—	32.0
75— 90	4.0	2.0	6.5	5.0	3.5	2.0	—	23.0
90—105	3.5	4.0	11.0	9.5	6.0	.5	—	34.5
105—120	3.5	—	.5	.5	1.0	.5	—	6.0
120—135	—	1.0	—	3.5	1.0	—	—	5.5
135—150	—	1.5	1.0	1.5	—	1.0	—	5.0
150—165	—	1.5	—	.5	1.0	—	—	3.0
165—180	—	2.0	—	.5	.5	—	—	3.0
180—195	—	1.0	—	2.5	.5	—	—	4.0
195—210	2.0	—	—	1.0	—	—	—	3.0
210—225	1.0	—	—	—	1.0	—	—	2.0
225—240	—	—	1.0	—	—	—	—	1.0
240—255	1.5	—	—	—	1.0	—	—	2.5
255—270	.5	—	1.0	—	—	—	—	1.5
270—285	1.0	—	—	—	—	—	—	1.0
Total	48.0	69.0	66.0	91.0	83.0	85.0	14.0	456.0

TABLE XXII.

*Average duration of plague in villages infected in different months.*

Month of 1st infection	No. of villages first reporting deaths in this month		Average No. of months in which deaths continued to be reported	
	Gujrat 1906-7	Hoshiarpur 1906-7	Gujrat 1906-7	Hoshiarpur 1906-7
December	16	97	5.44	2.96
January	20	122	4.45	2.98
February	56	143	3.91	2.69
March	165	224	3.25	2.18
April	294	192	2.67	1.63
May	223	90	2.04	1.28
June	67	20	1.28	1.10
July	9	4	1.00	1.00

TABLE XXII A.

*Death rates of villages reporting deaths in certain sequences.*

District and year	Sequence January, February, March			Sequence February, March, April		
	Population	Deaths	Rate	Population	Deaths	Rate
Mozaffarnagar 1907	7135	621	8.70	23,072	1532	6.64
Amritsar 1907	7017	345	4.92	8385	397	4.73
Amritsar 1905	18,177	743	4.09	30,930	1272	4.11
Hoshiarpur 1905	25,424	894	3.52	21,878	658	3.01
Hoshiarpur 1907	15,317	621	4.05	21,757	966	4.44
Total	73,070	3224	4.41	106,022	4825	4.55

TABLE XXIII.

*Particulars of epidemics in which over 300 villages were infected in any district.*

District and year	Particulars for all villages infected						Infected villages over 300 population						Infected villages under 300 population					
	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages		Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages		Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	
<b>Hoshiarpur :</b>																		
1902	404,430	476	11,688	2·89	849·6		63,755	12	864	1·36	5312·9		340,675	464	10,824	3·18	734·2	
1904	572,120	856	26,067	4·56	668·4		65,952	12	1425	2·16	5496·0		506,168	814	24,642	4·87	599·7	
1905	619,241	952	19,131	3·09	650·5		63,305	12	1052	1·66	5275·4		555,936	940	18,079	3·25	591·4	
1907	598,384	892	15,707	2·63	670·8		68,079	13	962	1·41	5236·8		530,305	879	14,745	2·78	603·3	
<b>Amritsar :</b>																		
1903	537,611	479	26,825	4·99	1122·4		104,317	25·5	3839	3·68	4090·8		433,294	453·5	22,986	5·30	955·4	
1904	509,119	429	18,372	3·61	1186·8		112,456	26·0	3184	2·83	4325·2		396,663	403·0	15,188	3·83	984·3	
1905	683,719	643	29,646	4·34	1063·3		130,630	31·5	4263	3·26	4147·0		553,089	611·5	25,383	4·59	904·5	
1907	633,670	590	23,642	3·73	1074·0		120,547	28·5	3010	2·50	4229·7		513,123	561·5	20,632	4·02	913·8	
<b>Gujrat :</b>																		
1904	277,151	305	23,785	8·58	908·7		69,671	9	849	1·22	7741·2		207,480	296	22,936	11·05	700·9	
1907	560,579	853	51,000	9·10	657·2		68,231	10	2718	3·98	6823·1		492,348	843	48,282	9·81	584·0	
<b>Mozaffarnagar :</b>																		
1905	545,689	316	16,246	2·98	1726·9		247,026	43	6961	2·82	5744·8		298,663	273	9285	3·11	1094·0	
1907	753,403	590	49,909	6·62	1277·0		264,202	49	16,365	6·19	5391·9		489,201	541	33,544	6·86	904·3	

TABLE XXIV.

Particulars of epidemics in which less than 300 villages were infected in any district.

District and year	Particulars for all villages infected					Infected villages over 3000 population					Infected villages under 3000 population				
	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages
<b>Hoshiarpur :</b>															
1898	17,438	16	439	2.52	1089.9	5803	1	87	1.50	5803.0	11,635	15	352	3.03	775.7
1899	3701	4	9	.24	925.3	—	—	—	—	—	3701	4	9	.24	925.3
1900	9636	11	50	.52	876.0	—	—	—	—	—	9636	11	50	.52	876.0
1901	42,790	45	441	1.04	950.9	9030	2	112	1.24	4515.0	33,760	43	332	.98	785.1
1903?	No data	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1906	228,728	293	4125	1.80	780.6	52,038	9	612	1.18	5782.0	176,690	284	3513	1.99	622.1
<b>Gujrat :</b>															
1902	72,200	38	551	.76	1900.0	37,875	3	14	.04	12,625.0	34,325	35	537	1.56	980.7
1903	87,724	58	1794	2.05	1512.5	50,902	6	673	1.32	8483.7	36,822	52	1121	3.04	708.1
1905	231,535	262	9782	4.22	883.7	46,619	5	775	1.66	9323.8	184,916	257	9007	4.87	719.5
1906	120,219	60	1207	1.00	2000.7	81,328	8	40	.05	10,166.0	38,891	52	1167	3.00	747.9
<b>Mozaffarnagar :</b>															
1902	25,581	2	4	.02	12,790.5	23,444	1	1	.00	23,444.0	2137	1	3	.14	2137.0
1903	73,944	23	782	1.06	3215.0	50,509	5	257	.51	10,101.8	23,435	18	525	2.24	1301.9
1904	257,455	128	5486	2.13	2011.4	131,227	20	2371	1.81	6561.4	126,228	108	3115	2.47	1168.8
1906	174,409	68	3077	1.76	2564.8	103,924	14	1526	1.47	7423.1	70,485	54	1551	2.20	1305.3
<b>Rohtak :</b>															
1904	147,918	56	2990	2.02	2641.4	108,513	17	1996	1.84	6383.1	39,405	39	994	2.52	1010.4
1905	491,746	284	28,802	5.86	1731.5	220,747	46	12,792	5.57	4994.5	261,999	238	16,010	6.11	1100.8
1906	121,872	31	1948	1.60	3931.4	97,761	16	1275	1.30	6110.1	24,111	15	673	2.79	1607.4
1907	469,355	247	31,330	6.68	1900.2	222,249	44	14,619	6.58	5096.6	247,106	203	16,711	6.76	1217.3
<b>Amritsar :</b>															
1902	97,154	58	1957	2.01	1675.1	34,514	7	651	1.89	4930.6	62,640	51	1306	2.08	1228.2
1906	339,770	272	6008	1.77	1249.2	90,985	21.5	1217	1.34	4231.9	248,785	250.5	4791	1.93	993.2

TABLE XXV.

*Plague experience in towns of over 13,500 inhabitants.*

Name of town	Population	Number of deaths	Mortality per cent.	Month of 1st infection	Number of months epidemic continued
Rohtak Town :					
1904	20,024	13	·065	February	4
1905	20,024	888	4·435	Oct. '04	8
1906	20,024	3	·015	April	2
1907	20,024	464	2·317	February	5
Amritsar City :					
1902	162,429	82	·051	February	Continuously infected.
1903	162,429	460	·283	Continuous	
1904	162,429	1103	·679	January	7
1905	162,429	1073	·661	January	20
1906	162,429	1903	1·172	Continuous	
1907	162,429	1101	·678	January	7
Mozaffarnagar :					
1902	23,444	1	·004	January	1
1903	23,444	25	·107	February	5
1904	23,444	331	1·412	Oct. '03	7
1905	23,444	249	1·062	Nov. '04	7
1906	23,444	112	·478	March	4
* 1907	23,444	490	2·090	Nov. '06	7
Kairana (Mozaffarnagar District) :					
1905	19,304	94	·487	January	6
1906	19,304	2	·010	April	1
1907	19,304	2136	11·065	February	5
Gujrat :					
1902	19,708	11	·056	May	2
1903	19,708	281	1·426	Aug. '02	12
1904	19,708	89	·452	Oct. '03	10
1905	19,708	183	·929	Oct. '04	9
1906	19,708	18	·091	April	2
1907	19,708	237	1·203	Oct. '06	10

\* One further case occurred here in Sept. 1907, three months after epidemic ceased.

TABLE XXVI.

HOSHIARPUR 1907.

*Infected villages having less than 1000 population, showing month of 1st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	32,914	74	1277	3·88	444·8
January	36,667	87	1529	4·17	421·5
February	53,767	117	2204	4·10	459·5
March	71,666	185	2681	3·74	387·4
April	65,994	167	1585	2·40	395·2
May	32,643	85	524	1·61	384·0
June	7486	16	86	1·15	467·9
July	1535	4	14	·91	383·8
Total	302,672	735	9900	3·27	411·8

*Infected villages having population over 1000 and under 3000, showing month of 1st infection.*

Dec. '06	40,689	25	1198	2·94	1627·6
January	43,073	29	1054	2·45	1485·3
February	28,723	19	865	3·01	1511·7
March	62,258	39	1256	2·02	1596·4
April	40,908	24	663	1·62	1704·5
May	7048	5	45	·64	1409·6
June	3945	2	13	·33	1972·5
July	1213	1	1	·03	1213·0
Total	227,857	144	5095	2·24	1582·3

TABLE XXVII.

AMRITSAR 1907.

*Infected villages having less than 1000 population, with month of 1st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	13,900	22	1011	7·27	631·8
January	9557	17	496	5·19	562·2
February	12,569	23	715	5·69	546·5
March	41,537	73	2837	6·83	569·0
April	66,540	122	3535	5·31	545·4
May	52,056	95	1793	3·44	548·0
June	9087	21	173	1·90	432·7
Total	205,246	373	10,560	5·15	550·3

*Villages with population from 1000—3000, and month of 1st infection.*

Dec. '06	26,407	17	1124	4·26	1553·4
January	12,699	9	482	3·80	1411·0
February	40,448	25	1481	3·66	1617·9
March	78,808	46	2816	3·57	1713·2
April	102,760	65	3406	3·31	1580·9
May	48,149	33	974	2·02	1459·1
June	8878	6	87	·98	1479·7
Total	318,149	201	10,370	3·26	1582·8

## Village Plague

TABLE XXVIII.

MOZAFFARNAGAR 1907.

*Infected villages having less than 1000 population, showing month of 1st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	10,292	16	375	3·64	643·3
January	9839	16	887	9·02	614·9
February	20,686	39	2315	11·19	530·4
March	42,767	77	4365	10·21	555·4
April	58,164	118	4026	6·92	492·9
May	35,079	68	1176	3·35	515·9
June	3304	7	68	2·06	472·0
Total	180,131	341	13,212	7·33	528·2

*Infected villages with population from 1000 to 3000, showing month of 1st infection.*

Dec. '06	32,425	18	1727	5·33	1801·4
January	41,076	25	3594	8·75	1643·0
February	41,792	27	3574	8·55	1547·9
March	77,593	48	6201	7·99	1616·5
April	71,259	47	4123	5·79	1516·1
May	38,740	26	631	1·63	1490·0
June	4102	3	56	1·37	1367·3
Total	306,987	194	19,906	6·48	1582·4

TABLE XXIX.

GUJRAT 1907.

*Infected villages with population up to 1000, showing month of 1st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	2494	5	264	10·59	498·8
January	8509	16	1252	14·71	531·8
February	18,364	43	2894	15·76	427·1
March	64,209	132	9144	14·24	486·4
April	103,341	243	12,726	12·31	425·3
May	76,499	203	4350	5·69	376·8
June	20,552	64	509	2·48	321·1
July	2402	8	34	1·42	300·3
Total	296,370	714	31,173	10·52	405·1

*Infected villages from 1000 to 3000 population, with month of 1st infection.*

Dec. '06	14,002	9	887	6·33	1555·8
January	7315	4	657	8·98	1828·8
February	19,353	12	2806	14·50	1612·8
March	47,987	31	5275	10·99	1548·0
April	73,958	48	6780	9·17	1540·8
May	26,498	21	634	2·39	1261·8
June	5462	3	54	·99	1820·7
July	1403	1	5	·36	1403·0
Total	195,978	129	17,098	8·72	1519·2

TABLE XXX.

ROHTAK 1907.

*Infected villages having less than 1000 population, showing month of 1st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	—	—	—	—	—
January	1082	2	53	4·90	541·0
February	6428	12	706	10·98	535·7
March	12,592	21	1050	8·34	599·6
April	17,688	31	1077	6·09	570·6
May	13,565	20	318	2·34	678·3
June	3091	5	20	·65	618·2
Total	54,446	91	3224	5·92	598·3

*Infected villages with populations from 1000 to 3000, showing month of 1st infection.*

Dec. '06	10,257	5	631	6·15	2051·4
January	4707	4	416	8·84	1176·8
February	7089	5	628	8·86	1417·8
March	85,310	47	8011	9·39	1815·1
April	64,589	38	3478	5·38	1699·7
May	19,348	12	300	1·55	1612·3
June	1360	1	23	1·69	1360·0
Total	192,660	112	13,487	7·00	1720·2

TABLE XXXI.

*Comparison of mortality in villages with population of under 300 with the mortality in those having a population of 300 or over in certain districts and epidemics.*

Population under 300.						
District and year	Infected first in month of	Total population	Number of villages	Total number of deaths	Mortality per cent.	Average population of villages
Gujrat 1907	April	17,627	92	2673	15·16	191·60
	May	16,445	88	1178	7·16	186·88
Hoshiarpur 1904	April	15,967	88	1429	8·95	181·44
	May	16,889	95	1013	6·00	177·78
Hoshiarpur 1907	March	15,099	82	892	5·91	184·13
	April	12,436	67	567	4·56	185·61
Population 300 or over.						
Gujrat 1907	April	173,047	202	17,071	9·86	856·67
	May	86,552	136	3806	4·40	636·41
Hoshiarpur 1904	April	120,786	161	5618	4·65	750·22
	May	110,711	148	3582	3·24	748·05
Hoshiarpur 1907	March	118,825	162	3045	2·56	733·49
	April	100,951	125	1681	1·67	807·61
All infected villages.						
Gujrat 1907	April	190,674	294	19,744	10·36	648·55
	May	102,997	224	4984	4·84	459·80
Hoshiarpur 1904	April	136,753	249	7047	5·15	549·10
	May	127,600	243	4595	3·60	525·10
Hoshiarpur 1907	March	133,924	224	3937	2·94	597·88
	April	113,387	192	2248	1·98	590·56



TABLE XXXII.

ROHTAK 1907.

Tehsil	Villages with population below 1000			Villages with population between 1000—3000		
	Total population	Deaths	Percentage	Total population	Deaths	Percentage
Rohtak	13,794	774	5·6	39,529	1547	3·9
Gohannah	11,434	686	6·0	69,668	5072	7·3
Sampla	20,052	1374	6·9	73,518	6665	9·1
Jhajjar	9166	390	4·3	9945	203	2·0

TABLE XXXIII.

*Proportions of villages in different groups infected in 1907\*.*

District	Mean population of villages 0—1000	Mean population of villages 1000—3000	Percentage No. of villages 0—1000 infected 1906—7	Percentage No. of villages 1000—3000 infected 1906—7	Ratio of percentage infections for the two groups	Ratio of mean populations for the two groups
Rohtak	458·5	1655·7	30·4	73·7	2·4	3·6
Amritsar	457·8	1539·3	47·7	81·7	1·7	3·4
Mozaffarnagar	413·4	1584·6	48·8	86·2	1·8	3·8
Hoshiarpur	334·5	1525·0	39·3	67·3	1·7	4·6

\* Gujrat not included owing to uncertainties respecting populations of non-infected villages.



TABLE XXXIV.

*Comparison of mortality rates including non-infected villages, 1907.*

Villages having less than 1000 population.					
District	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Rohtak	137,080	299	3224	2·35	458·5
Amritsar	357,961	782	10,560	2·95	457·8
Mozaffarnagar	288,964	699	13,212	4·57	413·4
Hoshiarpur	625,822	1871	9900	1·58	334·5
Villages having population of 1000—3000.					
Rohtak	251,662	152	13,487	5·36	1655·7
Amritsar	378,657	246	10,370	2·74	1539·3
Mozaffarnagar	356,528	225	19,906	5·58	1584·6
Hoshiarpur	326,359	214	5095	1·56	1525·0

TABLE XXXV.

GUJRAT 1907.

Villages infected in 2 months :						
	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Up to 1000	81,009	8476	224	10·46	2·24	361·6
1000—3000	31,925	1495	24	4·68	—	1330·2
Villages infected in 3 months :						
Up to 1000	107,763	12,424	232	11·53	1·16	464·5
1000—3000	72,732	7238	51	9·95	—	1426·1
Villages infected in 4 months :						
Up to 1000	49,097	6316	93	12·86	1·17	427·9
1000—3000	43,800	4811	27	10·98	—	1622·2

HOSHIARPUR 1907.

Villages infected 2 months :						
Up to 1000	64,553	2158	149	3·34	1·87	433·2
1000—3000	39,269	704	26	1·79	—	1510·3
Villages infected 3 months :						
Up to 1000	59,744	3070	132	5·14	2·32	352·6
1000—3000	46,229	1019	29	2·22	—	1594·1
Villages infected 4 months :						
Up to 1000	27,897	1642	58	5·89	1·86	481·0
1000—3000	42,447	1341	28	3·16	—	1516·0

TABLE XXXVI.

AMRITSAR 1907.

Villages infected during 2 months :						
	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Up to 1000	78,136	2998	156	3·84	2·48	500·9
1000—3000	62,717	971	45	1·55	—	1393·7
Villages infected during 3 months :						
Up to 1000	65,628	3735	113	5·69	1·64	580·8
1000—3000	115,058	3980	71	3·46	—	1620·5
Villages infected during 4 months :						
Up to 1000	26,655	2478	42	9·30	2·27	634·6
1000—3000	61,601	2527	38	4·10	—	1621·1

TABLE XXXVII.

MOZAFFARNAGAR 1907.

Villages infected 2 months :						
	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Up to 1000	60,531	3956	116	6·54	1·53	521·8
1000—3000	58,216	2486	39	4·27	—	1492·7
Villages infected 3 months :						
Up to 1000	48,425	5527	82	11·41	1·58	590·5
1000—3000	97,987	7069	65	7·21	—	1507·5
Villages infected 4 months :						
Up to 1000	15,923	1830	28	11·49	1·31	568·7
1000—3000	66,720	5840	39	8·75	—	1710·8

TABLE XXXVIII.

ROHTAK 1907.

Villages infected 2 months :						
	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Up to 1000	21,834	1212	34	5·55	1·67	642·2
1000—3000	41,819	1394	25	3·33	—	1672·8
Villages infected 3 months :						
Up to 1000	16,781	1228	28	7·32	·83	599·3
1000—3000	79,156	6984	48	8·82	—	1649·1
Villages infected 4 months :						
Up to 1000	4279	483	6	11·29	1·35	713·2
1000—3000	45,436	3800	23	8·36	—	1975·5

TABLE XXXIX.

General comparison of climatic conditions in the different districts, five months January—May (inclusive).

Year	Amritsar				Rohtak				Gujrat				Hoshiarpur				Mozaffarnagar			
	Mean mortality of villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Mean mortality per cent. Villages less than 3000 population	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Mean mortality per cent. Villages less than 3000 population	Average number of wet days	Mean mortality per cent. Villages less than 3000 population
1902	2.08	1.511	4.125	—	1.342	2.20	1.56	2.42	7.67	3.18	2.77	7.80	3.18	2.77	7.80	.14	.648	1.50		
1903	5.03	4.583	6.625	—	.528	2.00	3.04	4.55	12.33	Nodata	13.144	11.00	Nodata	13.144	11.00	2.24	1.095	3.17		
1904	3.83	6.430	13.50	2.52	2.222	5.80	11.05	9.47	15.33	4.87	7.036	15.00	4.87	7.036	15.00	2.47	4.300	9.67		
1905	4.59	4.429	11.875	6.11	3.10	7.00	4.87	6.96	13.33	3.25	8.37	16.40	3.25	8.37	16.40	3.11	3.720	8.67		
1906	1.93	6.264	12.875	2.79	3.022	4.60	3.00	8.643	14.00	1.99	6.364	14.40	1.99	6.364	14.40	2.20	5.203	9.50		
1907	4.02	7.429	17.00	6.85	4.144	8.00	9.81	14.803	22.00	2.78	15.022	22.40	2.78	15.022	22.40	6.86	6.870	12.67		

Comparison of mean daily temperature of Lahore with plague mortality in Amritsar district.

Year	January				February				March				April				May			
	Mean mortality of villages where epidemic com- menced in Jan.	Mean daily tem- perature	Mean daily range	Mean mortality of villages where epidemic com- menced in Feb.	Mean daily tem- perature	Mean daily range	Mean mortality of villages where epidemic com- menced in Mar.	Mean daily tem- perature	Mean daily range	Mean mortality of villages where epidemic com- menced in April	Mean daily tem- perature	Mean daily range	Mean mortality of villages where epidemic com- menced in May	Mean daily tem- perature	Mean daily range					
1903	5.26	55.1	27.7	6.00	59.9	27.5	5.79	66.8	24.6	5.19	76.9	28.6	2.84	87.2	28.9					
1904	3.81	53.1	22.3	6.15	60.4	30.0	5.47	67.1	19.3	3.56	81.1	30.5	1.32	91.0	28.4					
1905	4.71	51.6	18.6	5.04	50.1	21.2	4.67	63.6	20.9	4.05	78.0	28.9	2.23	92.7	29.4					
1906	2.89	53.1	28.9	2.60	56.8	19.1	2.58	64.9	21.7	2.41	78.5	28.7	.721	92.9	31.2					
1907	4.22	58.7	26.3	4.23	56.5	18.8	4.33	64.2	22.8	3.62	77.2	24.1	2.79	87.5	29.3					
Mean daily temperature, five months January—May (inclusive), Lahore																				
" " range																				
Mean percentage mortality for epidemics in Amritsar district																				
1903 1904 1905 1906 1907																				
69.18 70.54 67.20 69.24 68.82																				
27.46 26.10 23.80 25.92 24.26																				
5.03 3.83 4.59 1.93 4.02																				

TABLE XL.

*Amritsar City. Monthly deaths and meteorological conditions.  
Feb.—July, 1904—1907.*

Month and Year	Number of deaths	Proportion of deaths in the whole 6 months	Rainfall	Number of days upon which rain fell	Mean temperature for the month (Lahore record)
February 1904	0	0	·10	1	60·4°
„ 1905	237	·311	1·05	5	50·1
„ 1906	146	·081	2·90	5	56·8
„ 1907	30	·027	2·50	7	56·5
March 1904	36	·047	4·13	10	67·1°
„ 1905	169	·221	·37	2	63·6
„ 1906	333	·184	1·75	5	64·9
„ 1907	125	·114	1·95	5	64·2
April 1904	359	·471	·06	0	81·1°
„ 1905	127	·166	0	0	78·0
„ 1906	656	·363	·45	2	78·5
„ 1907	417	·382	1·20	3	77·2
May 1904	237	·311	·25	2	91·0°
„ 1905	170	·223	0	0	92·7
„ 1906	581	·321	·20	1	92·9
„ 1907	408	·374	·53	2	87·5
June 1904	109	·143	1·80	2	95·7°
„ 1905	48	·063	·25	1	96·4
„ 1906	83	·046	1·90	3	93·6
„ 1907	111	·102	·60	2	92·0
July 1904	22	·029	·10	1	93·0°
„ 1905	12	·016	3·55	6	92·1
„ 1906	9	·005	3·70	5	93·2
„ 1907	1	·001	1·80	4	93·0

TABLE XLI. Hoshiarpur District 1902.

HOSHIARPUR TEHSIL.							DASUYAH TEHSIL.						
Month of infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages		Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	
Dec. '01	21,987	1049	4.77	18	1221.5		Dec. '01	1133	1	.09	1	1133.0	
January	29,118	855	2.94	16	1819.9		January	861	2	.23	2	430.5	
February	30,473	1053	3.46	33	923.4		February	1294	23	1.78	1	1294.0	
March	23,224	409	1.76	26	893.2		March	6534	138	2.11	7	933.4	
April	22,317	363	1.63	24	929.9		April	10,017	341	3.40	4	2504.3	
May	10,040	140	1.39	19	528.4		May	4040	9	.22	2	2020.0	
June	905	3	.33	2	452.5		June	—	—	—	—	—	
July	2219	36	1.62	4	554.7		July	2543	15	.59	2	1271.5	
Total	140,283	3908	2.79	142	987.9		Total	26,422	529	2.00	19	1390.6	
GARSHANKAR TEHSIL.							UNA TEHSIL.						
Dec. '01	74,722	3233	4.33	93	803.5		Dec. '01	6550	260	3.97	4	1637.5	
January	51,784	1832	3.54	69	750.5		January	2537	31	1.22	2	1268.5	
February	30,480	979	3.21	58	525.5		February	9569	287	3.00	6	1594.8	
March	24,853	355	1.43	35	710.1		March	12,862	55	.43	11	1169.3	
April	11,959	176	1.47	20	597.9		April	3625	16	.44	4	906.3	
May	7040	23	.33	10	704.0		May	1744	4	.23	3	581.3	
June	—	—	—	—	—		June	—	—	—	—	—	
July	—	—	—	—	—		July	—	—	—	—	—	
Total	200,838	6598	3.29	285	704.7		Total	36,887	653	1.77	30	1229.6	
TOTAL HOSHIARPUR DISTRICT 1902.													
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages								
Dec. '01	104,392	4543	4.35	116	899.9								
January	84,300	2720	3.23	89	947.2								
February	71,816	2342	3.26	98	732.8								
March	67,473	957	1.42	79	854.1								
April	47,918	896	1.87	52	921.5								
May	22,864	176	.77	34	672.5								
June	905	3	.33	2	452.5								
July	4762	51	1.07	6	793.7								
Total	404,430	11,688	2.89	476	849.6								

TABLE XLII. *Hoshiarpur District 1904.*

HOSHIARPUR TEHSIL.						DASUYAH TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	—	—	—	—	—	Dec. '03	—	—	—	—	—
January	21,760	828	3·81	13	1673·8	January	20,963	1097	5·23	31	676·2
February	32,861	1652	5·03	20	1643·0	February	38,483	2722	7·07	43	895·0
March	30,053	1716	5·71	25	1202·1	March	23,612	1317	5·58	43	549·1
April	25,206	1466	5·82	50	504·1	April	42,048	2474	5·88	99	424·7
May	60,904	2466	4·05	101	603·0	May	26,265	971	3·70	64	410·4
June	14,147	202	1·43	32	442·1	June	4384	88	2·01	10	438·4
July	5028	216	4·30	8	628·5	July	414	2	·48	2	207·0
Total	189,959	8546	4·50	249	762·9	Total	156,169	8671	5·55	292	534·8
GARSHANKAR TEHSIL.						UNA TEHSIL.					
Dec. '03	—	—	—	—	—	Dec. '03	—	—	—	—	—
January	18,190	530	2·91	15	1212·7	January	1987	51	2·57	2	993·5
February	23,414	1410	6·02	27	867·2	February	1433	21	1·47	2	716·5
March	45,497	2363	5·19	64	710·9	March	11,120	49	·44	4	2780·0
April	62,050	3041	4·90	91	681·9	April	7449	66	·89	9	827·7
May	31,132	986	3·17	65	479·0	May	9299	172	1·85	13	715·3
June	11,645	156	1·34	20	582·3	June	—	—	—	—	—
July	495	2	·40	1	495·0	July	2281	3	·13	2	1140·5
Total	192,423	8488	4·41	283	679·9	Total	33,569	362	1·08	32	1049·0



TABLE XLIII. Hoshiarpur District 1905.

HOSHIAHPUR TEHSIL.						DASUYAH TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	46,628	2299	4.93	64	728.6	Dec. '04	12,742	611	4.80	13	980.2
January	76,700	2532	3.30	71	1080.3	January	42,372	1333	3.15	65	651.9
February	24,660	541	2.19	34	725.3	February	18,550	484	2.61	24	772.9
March	22,719	615	2.71	42	540.9	March	15,957	443	2.78	24	664.9
April	22,368	559	2.50	47	475.9	April	16,477	481	2.92	42	392.3
May	15,479	245	1.58	28	552.8	May	15,399	360	2.34	33	466.6
June	8189	129	1.58	19	431.0	June	13,595	204	1.50	28	485.5
July	1566	4	.26	3	522.0	July	1781	24	1.35	7	254.4
Total	218,309	6924	3.17	308	708.8	Total	136,873	3940	2.88	236	580.0

GARSHANKAR TEHSIL.						UNA TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	616	12	1.95	3	205.3	Dec. '04	14,892	593	3.98	21	709.1
January	58,570	2192	3.74	75	780.9	January	17,128	439	2.56	19	901.5
February	31,535	1169	3.71	43	733.4	February	14,668	228	1.55	19	772.0
March	35,327	1403	3.97	62	569.8	March	9747	135	1.39	18	541.5
April	50,748	1394	2.75	76	667.7	April	12,115	250	2.06	29	417.8
May	16,969	356	2.10	32	530.3	May	3161	47	1.49	9	351.2
June	7558	102	1.35	20	377.9	June	1185	1	.08	1	1185.0
July	1356	13	.96	3	452.0	July	2841	48	1.69	3	947.0
Total	202,679	6641	3.28	314	645.5	Total	75,737	1741	2.30	119	636.4

TOTAL HOSHIAHPUR DISTRICT 1905.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	74,878	3515	4.69	101	741.4
January	194,770	6496	3.34	230	846.8
February	89,413	2422	2.71	120	745.1
March	83,750	2596	3.10	146	573.6
April	101,708	2684	2.64	194	524.6
May	51,008	1008	1.98	102	500.1
June	30,527	436	1.43	68	448.9
July	7544	89	1.18	16	471.5
Total	633,598	19,246	3.04	977	618.5

TABLE XLIV. *Hoshiarpur District 1907.*

HOSHIARPUR TEHSIL.						DASUYAH TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	21,134	639	3.02	25	845.4	Dec. '06	23,510	875	3.72	40	587.8
January	46,257	1233	2.67	35	1321.6	January	36,717	976	2.66	42	874.2
February	24,215	894	3.69	43	563.1	February	18,093	736	4.07	37	489.0
March	44,635	863	1.93	67	666.2	March	25,716	1142	4.44	56	459.2
April	37,029	527	1.42	55	673.3	April	27,765	750	2.70	50	555.3
May	16,155	172	1.06	33	489.5	May	4963	105	2.12	18	275.7
June	12,469	82	.66	10	1246.9	June	2094	11	.53	6	349.0
July	619	2	.32	2	309.5	July	1213	1	.08	1	1213.0
Total	202,513	4412	2.18	270	750.0	Total	140,071	4596	3.28	250	560.3
GARSHANKAR TEHSIL.						UNA TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	26,304	815	3.10	31	848.5	Dec. '06	2443	146	5.98	3	814.3
January	35,858	1096	3.07	43	833.9	January	976	41	4.20	3	325.3
February	42,704	1221	2.86	53	805.7	February	11,972	218	1.82	6	1995.3
March	46,321	1382	2.98	80	579.0	March	17,252	550	3.19	21	821.5
April	32,102	661	2.06	63	509.6	April	16,491	310	1.88	24	687.1
May	7744	182	2.35	16	484.0	May	10,829	110	1.02	23	470.8
June	1639	3	.18	2	819.5	June	2261	12	.53	2	1330.5
July	497	11	2.21	1	497.0	July	419	1	.24	1	419.0
Total	193,169	5371	2.78	289	668.4	Total	62,643	1388	2.22	83	754.7

TOTAL HOSHIARPUR DISTRICT 1907.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	73,391	2475	3.37	99	741.3
January	119,808	3346	2.79	123	974.0
February	96,984	3069	3.16	139	697.7
March	133,924	3937	2.94	224	597.9
April	113,387	2248	1.98	192	590.6
May	39,691	569	1.43	90	441.0
June	18,463	108	.58	20	923.2
July	2748	15	.55	5	549.6
Total	598,396	15,767	2.63	892	670.8

TABLE XLV. Amritsar 1903.

AMRITSAR TEHSIL.						TARN-TARN TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '02	31,758	1491	4.69	25	1270.3	Dec. '02	34,237	2696	7.87	23	1488.6
January	39,150	1847	4.72	40	978.8	January	28,512	1741	6.11	26	1096.6
February	27,168	1312	4.83	21	1293.7	February	39,403	2823	7.16	36	1094.5
March	45,033	2440	5.42	35	1286.7	March	37,244	2368	6.36	33	1128.6
April	8055	522	6.48	8	1006.9	April	38,745	2430	6.27	39	993.5
May	45,835	1653	3.61	41	1117.9	May	26,066	425	1.63	21	1241.2
June	4190	90	2.15	6	698.3	June	—	—	—	—	—
Total	201,189	9355	4.65	176	1143.1	Total	204,207	12,483	6.11	178	1147.2

AJNALA TEHSIL.						TOTAL AMRITSAR DISTRICT 1903.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '02	3367	137	4.07	4	841.8	Dec. '02	69,362	4324	6.23	52	1333.9
January	4228	194	4.59	5	704.7	January	71,890	3782	5.26	71	1012.5
February	17,987	936	5.20	15	1199.1	February	84,558	5071	6.00	72	1174.4
March	22,362	1161	5.19	27	828.2	March	104,639	5969	5.79	95	1101.5
April	42,923	1705	3.97	39	1100.6	April	89,723	4657	5.19	86	1043.3
May	17,307	455	2.63	24	721.1	May	89,208	2533	2.84	86	1037.3
June	5246	104	1.98	8	655.8	June	9436	194	2.06	14	674.0
Total	113,420	4692	4.14	122	929.7	Total	518,816	26,530	5.11	476	1089.9

TABLE XLVI. Amritsar 1904.

AMRITSAR TEHSIL.						TARN-TARN TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	1700	31	1.82	1	1700.0	Dec. '03	—	—	—	—	—
January	26,560	1053	3.96	13	2043.1	January	15,902	686	4.31	6	2650.3
February	17,224	1115	6.47	12	1435.3	February	8514	580	6.81	7	1216.3
March	47,491	2897	6.10	40	1187.3	March	31,521	1597	5.07	20	1576.1
April	84,713	3080	3.64	93	910.9	April	102,974	3998	3.88	80	1287.2
May	37,497	547	1.46	37	1013.4	May	38,789	418	1.08	44	881.6
June	1808	9	.50	3	602.7	June	71	1	1.41	1	71.0
Total	216,993	8732	4.02	199	1090.4	Total	197,771	7280	3.68	158	1251.7
AJNALA TEHSIL.						TOTAL AMRITSAR DISTRICT 1904.					
Dec. '03	2179	25	1.15	1	2179.0	Dec. '03	3879	56	1.44	2	1939.5
January	2558	4	.16	1	2558.0	January	45,020	1743	3.87	20	2250.0
February	8469	408	4.82	4	2117.3	February	34,207	2103	6.15	23	1487.3
March	10,284	392	3.81	10	1028.4	March	89,296	4886	5.47	70	1275.7
April	36,239	902	2.49	34	1065.9	April	223,926	7980	3.56	207	1081.8
May	15,851	252	1.59	19	834.3	May	92,137	1217	1.32	100	921.4
June	—	—	—	—	—	June	1879	10	.53	4	469.8
Total	75,580	1983	2.62	69	1095.4	Total	490,344	17,995	3.67	426	1151.0

Amritsar 1905.

TABLE XLVII.

AMRITSAR TEHSIL.							TARN-TARN TEHSIL.						
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages		Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	
Dec. '04	49,893	2524	5.06	38	1313.0		Dec. '04	47,944	2994	6.24	28	1712.4	
January	75,278	3489	4.63	71	1060.3		January	34,212	1857	5.43	37	924.7	
February	46,119	2090	4.53	47	981.3		February	50,381	2747	5.45	40	1259.5	
March	52,869	2161	4.09	55	961.3		March	64,179	3769	5.87	56	1146.1	
April	35,318	1055	2.99	36	981.1		April	55,319	2132	3.85	55	1005.8	
May	11,658	211	1.81	14	832.7		May	26,025	584	2.24	31	839.5	
June	—	—	—	—	—		June	—	—	—	—	—	
Total	271,135	11,530	4.25	261	1038.8		Total	278,060	14,083	5.06	247	1125.7	
AJNALA TEHSIL.							TOTAL AMRITSAR DISTRICT 1905.						
Dec. '04	12,531	319	2.55	9	1392.3		Dec. '04	110,368	5837	5.29	75	1471.5	
January	10,896	351	3.22	10	1089.6		January	120,386	5697	4.73	118	1020.2	
February	5658	316	5.59	8	707.3		February	102,158	5153	5.04	95	1075.3	
March	23,362	738	3.16	18	1297.9		March	140,410	6668	4.75	129	1088.4	
April	28,596	1474	5.15	35	817.0		April	119,233	4661	3.91	126	946.3	
May	39,715	925	2.33	49	810.5		May	77,398	1720	2.22	94	823.4	
June	2855	38	1.33	6	475.8		June	2855	38	1.33	6	475.8	
Total	123,613	4161	3.37	135	915.7		Total	672,808	29,774	4.43	643	1046.4	

TABLE XLVIII. Amritsar 1906.

AMRITSAR TEHSIL.						TARN-TARN TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	2463	68	2.76	2	1231.5	Dec. '05	—	—	—	—	—
January	8710	211	2.42	7	1244.3	January	9987	330	3.30	5	1997.4
February	12,299	353	2.87	7	1757.0	February	2208	25	1.13	1	2208.0
March	40,737	1335	3.28	25	1629.5	March	17,674	213	1.21	11	1606.7
April	66,272	1809	2.73	72	920.4	April	28,637	449	1.57	22	1301.7
May	51,000	407	.80	53	962.3	May	33,609	158	.47	27	1244.8
June	11,359	69	.61	9	1262.1	June	6694	6	.09	5	1338.8
Total	192,840	4252	2.20	175	1101.9	Total	98,809	1181	1.20	71	1391.7
AJNALA TEHSIL.						TOTAL AMRITSAR DISTRICT 1906.					
Dec. '05	—	—	—	—	—	Dec. '05	2463	68	2.76	2	1231.5
January	—	—	—	—	—	January	18,697	541	2.89	12	1558.1
February	1455	37	2.54	2	727.5	February	15,962	415	2.60	10	1596.2
March	6542	128	1.96	2	3271.0	March	64,953	1676	2.58	38	1709.3
April	2100	86	4.10	3	700.0	April	97,009	2344	2.42	97	1000.1
May	11,554	128	1.11	12	962.8	May	96,163	693	.72	92	1045.3
June	3914	3	.08	2	1957.0	June	21,967	78	.36	16	1372.9
Total	25,565	382	1.49	21	1217.4	Total	317,214	5815	1.83	267	1188.1

TABLE XLIX. Amritsar 1907.

AMRITSAR TEHSIL.						TARN-TARN TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	22,742	1222	5.37	19	1196.9	Dec. '06	5531	234	4.23	4	1382.8
January	25,491	992	3.89	19	1341.6	January	5800	181	3.12	4	1450.0
February	24,633	884	3.59	25	985.3	February	19,570	752	3.84	12	1630.8
March	78,868	2848	3.61	70	1126.7	March	44,626	1795	4.02	30	1487.5
April	59,139	2141	3.62	60	985.7	April	95,592	2232	2.33	73	1309.5
May	23,476	392	1.67	30	782.5	May	42,136	930	2.21	49	859.9
June	5976	73	1.22	8	747.0	June	5074	57	1.12	7	724.9
Total	240,325	8552	3.56	231	1040.4	Total	218,329	6181	2.83	179	1219.7
AJNALA TEHSIL.						TOTAL AMRITSAR DISTRICT 1907.					
Dec. '06	13,210	766	5.80	9	1467.8	Dec. '06	41,483	2222	5.36	32	1296.3
January	6092	403	6.62	9	676.9	January	37,383	1576	4.22	32	1168.2
February	16,520	932	5.64	13	1270.8	February	60,723	2568	4.23	50	1214.5
March	26,173	1830	6.99	25	1046.9	March	149,667	6473	4.33	125	1197.3
April	51,679	3091	5.98	62	833.5	April	206,410	7464	3.62	195	1058.5
May	37,506	1550	4.13	49	765.4	May	103,118	2872	2.79	128	805.6
June	5041	120	2.38	10	504.1	June	16,091	250	1.55	25	643.6
Total	156,221	8692	5.56	177	882.6	Total	614,875	23,425	3.81	587	1047.5

TABLE L. Rohtak 1905.

ROHTAK TEHSIL.						SAMPLA TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	24,244	815	3.36	6	4040.7	Dec. '04	15,999	889	5.56	6	2666.5
January	61,097	3904	6.39	19	3215.6	January	21,989	1668	7.59	10	2198.9
February	11,293	868	7.69	10	1129.3	February	38,694	2476	6.40	21	1842.6
March	22,766	1940	8.52	14	1626.1	March	21,022	1916	9.11	17	1236.6
April	27,646	801	2.90	22	1256.6	April	24,921	1225	4.92	19	1311.6
May	1886	36	1.91	2	943.0	May	5428	50	.92	5	1085.6
June	—	—	—	—	—	June	1095	8	.73	1	1095.0
Total	148,932	8364	5.62	73	2040.2	Total	129,148	8232	6.37	79	1634.8
JHAJAR TEHSIL.						GOHANAH TEHSIL.					
Dec. '04	12,657	599	4.73	9	1406.3	Dec. '04	10,720	1110	10.35	4	2680.0
January	6631	679	10.24	9	736.8	January	8262	1136	13.75	3	1301.3
February	4949	282	5.70	8	618.6	February	12,618	962	7.62	4	3154.5
March	17,165	889	5.18	17	1009.7	March	21,843	2335	10.69	9	2427.0
April	16,481	648	3.93	25	659.2	April	46,779	2089	4.47	30	1559.3
May	6557	81	1.24	4	1639.3	May	16,753	234	1.40	8	2094.1
June	—	—	—	—	—	June	—	—	—	—	—
Total	64,440	3178	4.93	72	895.0	Total	116,975	7866	6.72	58	2016.8

TOTAL ROHTAK DISTRICT 1905.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	63,620	3413	5.36	25	2544.8
January	97,979	7387	7.54	41	2389.7
February	67,554	4588	6.79	43	1571.0
March	82,796	7080	8.55	57	1452.6
April	115,827	4763	4.11	96	1206.5
May	30,624	401	1.31	19	1611.8
June	1095	8	.73	1	1095.0
Total	459,495	27,640	6.02	282	1629.4



TABLE LI. Rohtak 1907.

ROHTAK TEHSIL.						SAMPLA TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	5126	234	4.56	1	5126.0	Dec. '06	6371	340	5.34	2	3185.5
January	14,724	1710	11.61	3	4908.0	January	8548	662	7.74	4	2137.0
February	14,113	1183	8.38	5	2822.6	February	15,170	934	6.16	7	2167.1
March	67,038	3282	4.90	23	2914.7	March	64,098	6917	10.79	40	1602.5
April	30,417	747	2.46	21	1448.4	April	27,130	2344	8.64	20	1356.5
May	10,330	103	1.00	7	1475.7	May	12,284	205	1.67	12	1023.7
June	612	1	.16	1	612.0	June	1685	25	1.48	2	842.5
Total	142,360	7260	5.10	61	2333.8	Total	135,286	11,427	8.45	87	1555.0
JHAJAR TEHSIL.						GOHANAH TEHSIL.					
Dec. '06	—	—	—	—	—	Dec. '06	26,632	3029	11.37	8	3329.0
January	—	—	—	—	—	January	6236	769	12.33	4	1559.0
February	839	108	12.87	1	839.0	February	26,956	2175	8.07	13	2073.5
March	2735	109	3.99	3	911.7	March	36,014	3491	9.69	19	1895.5
April	10,776	306	2.84	14	769.7	April	28,028	1823	6.50	18	1557.1
May	8500	68	.80	7	1214.3	May	11,786	267	2.27	9	1309.6
June	1063	6	.56	2	531.5	June	930	11	1.18	1	930.0
Total	23913	597	2.50	27	885.7	Total	136,582	11,565	8.47	72	1897.0

TOTAL ROHTAK DISTRICT 1907.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	38,129	3603	9.45	11	3466.3
January	29,508	3141	10.64	11	2682.5
February	57,078	4400	7.71	26	2195.3
March	169,885	13,799	8.12	85	1998.6
April	96,351	5220	5.42	73	1319.9
May	42,900	643	1.50	35	1225.7
June	4290	43	1.00	6	715.0
Total	438,141	30,849	7.04	247	1773.9



TABLE LIII. *Mozaffarnagar* 1905.

MOZAFFARNAGAR TEHSIL.						JANSATH TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	42,302	1743	4.12	16	2643.9	Dec. '04	83,558	3106	3.72	39	2142.5
January	27,246	1100	4.04	14	1946.1	January	36,196	1051	2.90	27	1340.6
February	25,801	879	3.41	15	1720.1	February	6448	134	2.08	9	716.4
March	46,657	976	2.09	37	1261.0	March	45,848	1377	3.00	28	1637.4
April	19,560	423	2.16	18	1086.7	April	18,302	496	2.71	16	1143.9
May	16,215	329	2.03	16	1013.4	May	9784	119	1.22	9	1087.1
June	1720	4	.23	3	573.3	June	1349	3	.22	1	1349.0
Total	179,501	5454	2.04	119	1508.4	Total	201,485	6286	3.12	129	1561.9
BUDIANA TEHSIL.						KAIRANA TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	12,128	126	1.04	7	1732.6	Dec. '04	—	—	—	—	—
January	12,720	586	4.61	5	2544.0	January	6376	275	4.31	2	3188.0
February	6287	349	5.55	4	1571.8	February	1245	40	3.21	1	1245.0
March	26,083	1372	5.26	13	2006.4	March	7966	183	2.30	4	1991.5
April	21,697	1016	4.68	13	1669.0	April	7555	130	1.72	7	1079.3
May	5320	78	1.47	6	886.7	May	3024	20	.66	3	1008.0
June	—	—	—	—	—	June	—	—	—	—	—
Total	84,235	3527	4.19	48	1754.9	Total	26,166	648	2.48	17	1539.2
TOTAL MOZAFFARNAGAR DISTRICT 1905.											
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	137,988	4975	3.61	62	2262.1	Dec. '04	137,988	4975	3.61	62	2262.1
January	82,538	3012	3.65	48	1719.5	January	82,538	3012	3.65	48	1719.5
February	39,781	1402	3.52	29	1371.8	February	39,781	1402	3.52	29	1371.8
March	126,554	3908	3.09	82	1543.3	March	126,554	3908	3.09	82	1543.3
April	67,114	2065	3.08	54	1242.9	April	67,114	2065	3.08	54	1242.9
May	34,343	546	1.59	34	1010.1	May	34,343	546	1.59	34	1010.1
June	3069	7	.23	4	767.3	June	3069	7	.23	4	767.3
Total	491,387	15,915	3.24	313	1569.9	Total	491,387	15,915	3.24	313	1569.9

TABLE LIV. *Mozaffarnagar* 1906.

MOZAFFARNAGAR TEHSIL.						JANSATH TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	3565	140	3.93	1	3565.0	Dec. '05	9597	144	1.50	3	3199.0
January	3428	214	6.24	2	1714.0	January	—	—	—	—	—
February	1448	2	.14	2	724.0	February	8888	588	6.62	3	2962.7
March	3540	88	2.49	3	1180.0	March	14,826	471	3.18	7	2118.0
April	5593	52	.93	4	1398.3	April	8085	136	1.68	6	1347.5
May	557	1	.18	1	557.0	May	5855	46	.79	3	1951.7
June	—	—	—	—	—	June	2699	1	.04	1	2699.0
Total	18,131	497	2.74	13	1394.7	Total	49,950	1386	2.77	23	2171.7
BUDHANA TEHSIL.						KAIRANA TEHSIL.					
Dec. '05	9651	177	1.83	3	3217.0	Dec. '05	2016	12	.60	2	1008.0
January	—	—	—	—	—	January	—	—	—	—	—
February	6973	424	6.08	3	2324.3	February	—	—	—	—	—
March	10,226	266	2.60	8	1278.3	March	—	—	—	—	—
April	12,154	65	.53	7	1736.3	April	1514	45	2.97	2	757.0
May	6789	36	.53	3	2263.0	May	2694	2	.07	1	2694.0
June	—	—	—	—	—	June	—	—	—	—	—
Total	45,793	968	2.11	24	1908.0	Total	6224	59	.95	5	1244.8

TOTAL MOZAFFARNAGAR DISTRICT 1906.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	24,829	473	1.91	9	2758.8
January	3428	214	6.24	2	1714.0
February	17,309	1014	5.86	8	2163.6
March	28,592	825	2.89	18	1588.4
April	27,346	298	1.09	19	1439.3
May	15,895	85	.53	8	1986.9
June	12699	1	.04	1	2699.0
Total	120,098	2910	2.42	65	1847.7

TABLE LV. Mozaffarnagar 1907.

MOZAFFARNAGAR TEHSIL.						JANSATH TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	24,248	920	3.79	11	2204.4	Dec. '06	55,280	2663	4.82	26	2126.2
January	24,516	2375	9.69	11	2228.7	January	39,613	3319	8.38	32	1237.9
February	46,588	3878	8.32	27	725.5	February	59,294	4534	7.65	40	1482.4
March	54,417	4865	8.94	52	1046.5	March	61,561	4820	7.83	60	1026.0
April	57,489	3396	5.91	71	809.7	April	35,311	2511	7.11	39	905.4
May	19,160	380	1.98	29	660.7	May	18,908	409	2.16	25	756.3
June	2144	16	.75	3	714.7	June	—	—	—	—	—
Total	228,562	15,830	6.93	204	1120.4	Total	269,967	18,256	6.76	222	1216.1
BUDHANA TEHSIL.						KAIRANA TEHSIL.					
Dec. '06	1791	116	6.48	2	895.5	Dec. '06	2261	154	6.81	2	1130.5
January	18,885	1509	7.99	5	3777.0	January	4120	295	7.16	2	2060.0
February	24,305	1819	7.48	12	2025.4	February	3047	291	9.55	2	1523.5
March	16,611	1751	10.54	11	1510.1	March	12,616	1590	12.60	10	1261.6
April	32,320	1512	4.68	26	1243.1	April	35,541	2246	6.32	38	935.3
May	29,727	628	2.11	24	1238.6	May	12,363	535	4.33	21	588.7
June	6317	84	1.33	4	1579.3	June	561	22	3.92	3	187.0
Total	129,956	7419	5.71	84	1547.1	Total	70,509	5133	7.28	78	903.4

TOTAL MOZAFFARNAGAR DISTRICT 1907.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	83,580	3746	4.61	41	2038.5
January	87,134	7498	8.61	50	1742.7
February	133,234	10,522	7.90	81	1644.9
March	145,205	13,026	8.97	133	1091.8
April	160,661	9665	6.02	174	923.3
May	80,158	1952	2.44	99	809.7
June	9022	122	1.35	10	902.2
Total	698,994	46,531	6.67	588	1188.8

TABLE LVI. *Gujrat* 1904.

GUJRAT TEHSIL.							KHARIAN TEHSIL.						
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages		Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	
Dec. '03	—	—	—	—	—		Dec. '03	5454	176	3.23	1	5454.0	
January	—	—	—	—	—		January	—	—	—	—	—	
February	667	39	5.85	2	333.5		February	1665	28	1.68	2	832.5	
March	16,813	710	4.22	12	1401.1		March	3509	137	3.90	5	701.8	
April	27,403	812	2.96	33	830.4		April	13,441	497	3.70	13	1033.9	
May	13,380	205	1.53	20	669.0		May	6182	246	3.98	10	618.2	
June	769	17	2.21	3	256.3		June	5854	30	.51	7	836.3	
July	—	—	—	—	—		July	620	1	.16	1	620.0	
Total	59,032	1783	3.02	70	843.3		Total	36,725	1115	3.04	39	941.7	
PHALIA TEHSIL.							TOTAL GUJRAT DISTRICT 1904.						
Dec. '03	8199	736	8.98	9	911.0		Dec. '03	13,653	912	6.68	10	1365.3	
January	3808	1051	27.60	4	952.0		January	3808	1051	27.60	4	952.0	
February	14,197	1723	12.14	10	1419.7		February	16,529	1790	10.83	14	1180.6	
March	37,810	8338	22.05	51	741.4		March	58,132	9185	15.80	68	854.9	
April	57,492	8410	14.63	97	592.7		April	98,336	9719	9.88	143	687.7	
May	11,006	391	3.55	16	687.9		May	30,568	842	2.76	46	664.5	
June	5704	97	1.70	6	950.7		June	12,327	144	1.17	16	770.4	
July	—	—	—	—	—		July	620	1	.16	1	620.0	
Total	138,216	20,746	15.01	193	716.1		Total	233,973	23,644	10.11	302	774.8	

TABLE LVII. *Gujrat* 1905.

GUJRAT TEHSIL.						KHARIAN TEHSIL.					
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	6923	668	9.65	10	692.3	Dec. '04	—	—	—	—	—
January	5645	432	7.65	12	470.4	January	302	3	.99	1	302.0
February	6377	537	8.42	9	708.6	February	1725	5	.29	2	862.5
March	27,821	1812	6.51	27	1030.4	March	807	265	32.84	2	403.5
April	28,671	2109	7.36	44	651.6	April	8959	372	4.15	11	814.5
May	26,875	494	1.84	34	790.4	May	7328	132	1.80	8	916.0
June	4062	21	.52	9	451.3	June	—	—	—	—	—
July	—	—	—	—	—	July	—	—	—	—	—
Total	106,374	6073	5.71	145	733.6	Total	19,121	777	4.06	24	796.7
PHALIA TEHSIL.						TOTAL GUJRAT DISTRICT 1905.					
Dec. '04	4436	153	3.45	4	1109.0	Dec. '04	11,359	821	7.23	14	811.4
January	3051	159	5.21	3	1017.0	January	8998	594	6.60	16	562.4
February	583	87	14.92	2	291.5	February	8685	629	7.24	13	668.1
March	19,852	598	3.01	23	863.1	March	48,480	2675	5.52	52	932.3
April	33,056	1216	3.68	38	869.9	April	70,686	3697	5.23	93	760.1
May	12,654	207	1.64	19	666.0	May	46,857	833	1.78	61	768.2
June	965	7	.73	2	482.5	June	5027	28	.56	11	457.0
July	—	—	—	—	—	July	—	—	—	—	—
Total	74,597	2427	3.25	91	819.7	Total	200,092	9277	4.64	260	769.6

TABLE LVIII. *Gujrat* 1907.

GUJRAT TEHSIL.							KHARIAN TEHSIL.						
Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages		Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages	
Dec. '06	7626	61	80	2	3813.0		Dec. '06	—	—	—	—	—	
January	—	—	—	—	—		January	—	—	—	—	—	
February	3569	350	9.81	5	713.8		February	2333	443	18.99	2	1166.5	
March	35,037	3022	8.63	45	778.6		March	23,614	3701	15.67	25	944.6	
April	84,250	6298	7.48	128	658.2		April	68,216	9216	13.50	100	682.2	
May	48,702	1899	3.90	101	482.2		May	51,912	2925	5.63	113	459.4	
June	16,208	289	1.78	32	506.5		June	9708	255	2.63	34	285.5	
July	570	21	3.68	3	190.0		July	3235	18	.56	6	539.2	
Total	195,962	11,940	6.09	316	620.1		Total	159,018	16,552	10.41	280	568.5	
PHALIA TEHSIL.							TOTAL GUJRAT DISTRICT 1907.						
Dec. '06	20,570	1680	8.17	14	1469.3		Dec. '06	28,196	1741	6.17	16	1762.3	
January	15,824	1909	12.06	20	791.2		January	15,824	1909	12.06	20	791.2	
February	34,934	5163	14.78	49	712.9		February	40,836	5956	14.59	56	729.2	
March	62,139	8576	13.80	95	654.1		March	120,790	15,299	12.67	165	732.1	
April	38,208	4236	11.09	66	578.9		April	190,674	19,744	10.36	294	648.6	
May	2383	160	6.71	10	238.3		May	102,997	4984	4.84	224	459.8	
June	98	19	19.39	1	98.0		June	26,014	563	2.16	67	388.3	
July	—	—	—	—	—		July	3805	39	1.03	9	422.8	
Total	174,156	21,743	12.48	255	682.7		Total	529,136	50,235	9.49	851	621.8	



TABLE LIX.

*Infected villages within selected area, Gujrat 1907.*

Month of 1st infection	Population	No. of deaths	No. of villages	Mortality per cent.	Average population of villages
Dec. '06	—	—	—	—	—
January	—	—	—	—	—
February	7394	1642	8	22·21	924·3
March	28,616	4992	27	17·44	1059·9
April	33,276	6503	53	19·54	627·8
May	11,692	1045	26	8·94	449·7
June	1425	89	8	6·25	178·1
Total	82,403	14,271	122	17·32	675·4

*Infected villages without selected area, Gujrat 1907.*

Dec. '06	28,196	1741	16	6·17	1762·3
January	15,824	1909	20	12·06	791·2
February	33,442	4314	48	12·90	696·7
March	92,174	10,307	138	11·18	667·9
April	157,398	13,241	241	8·41	653·1
May	91,305	3939	198	4·31	461·1
June	24,589	474	59	1·93	416·8
July	3805	39	9	1·02	422·8
Total	446,733	35,964	729	8·05	612·8

TABLE LX.

*Infected villages within selected area, Gujrat District 1904.*

Month of 1st infection	Population	No. of deaths	No. of villages	Mortality per cent.	Average population of villages
Dec. '03	5454	176	1	3·23	5454·0
January	—	—	—	—	—
February	534	18	1	3·37	534·0
March	689	209	3	30·33	229·7
April	15,146	1215	15	8·02	1009·7
May	3972	205	6	5·16	662·0
June	3383	18	4	·53	845·8
July	—	—	—	—	—
Total	29,178	1841	30	6·31	972·6

*Infected villages without selected area, Gujrat District 1904.*

Dec. '03	8199	736	9	8·98	911·0
January	3808	1051	4	27·60	952·0
February	15,995	1772	13	11·08	1230·4
March	57,443	8976	65	15·63	883·7
April	83,190	8504	128	10·22	649·9
May	26,596	637	40	2·40	664·9
June	8944	126	12	1·41	745·3
July	620	1	1	·16	620·0
Total	204,795	21,803	272	10·65	752·9

TABLE LXI. *Clerical mortality 1348—9.*

## (1) INSTITUTIONS AND AVERAGE INSTITUTIONS.

Place		Average No. of Institutions (Annual)	Institutions 1348—9	Ratio
Cornwall & Devon	...	36	382	10·6
Hants.	... ..	21	228	10·9
Cambridge	... ..	9	97	10·8
Hereford	... ..	13	200	15·4
Shropshire	... ..	1½	29	19·3
Derby	... ..	7	77 (deaths)	11·0
Wilts.	... ..	26	176 (in 2 years)	6·8
Norwich (Diocese)	...	35	297 (in 2 years)	8·5
Bath & Wells (Diocese)		77	800	10·3

## (2) TOTAL BENEFICES AND INSTITUTIONS.

				Percentage
Bucks.	... ..	180	83	46·1
Worcester	... ..	138	67	48·5
Northants.	... ..	281	161	57·3
Derby	... ..	108	77	71·3
Dorset	... ..	211	110	52·1
Notts.	... ..	126	65	51·6
Chester	... ..	70	30 (1 Archdeaconry)	—
W. R. Yorks.	...	141	96	68·1
E. R. Yorks. ...	...	126	65	51·6

TABLE LXII. *English Records.*

EPIDEMIC OF 1603—4.			
City or village	Estimated population	Population required to give the same rate of mortality as London	Plague deaths
London	250,000	250,000	33,347
Chester	Less than 6000 *	12,265	1636
York	Less than 6000 †	26,329	3512
Shrewsbury	Less than 4000 ‡	5000	667
EPIDEMIC OF 1625—6.			
London	320,000	320,000	41,313
Plymouth	Less than 10,000 *	15,492	2000
Ashburton	—	2827	365 §
Norwich	23,000	11,085	1431
EPIDEMIC OF 1665—6.			
London	460,000	460,000	68,596
Eyam	350	1737	259
Deptford	—	3500	522
Colchester	Less than 10,000 *	32,302	4817

\* "In the reign of Charles the Second no provincial town in the kingdom contained thirty thousand inhabitants and only four provincial towns as many as ten thousand inhabitants." Macaulay, i. 164.

(The four towns were Bristol, Norwich, York, Exeter.)

† Macaulay estimates the population in 1685 at about 10,000.

‡ 7000 inhabitants at the census of 1695.

§ "Probably a fourth of the inhabitants," *Notes and Queries*, 6 Ser. III. 477, cited by Creighton, i. 524.

|| 28,000—29,000 at the census of 1693.

TABLE LXIII.

*The plague experience of the South of France 1720—21.*

Population group	No. of towns	Total population	Total deaths	Death rate
90,000	1 (Marseilles)	90,000	39,134	43·5
20,000—90,000	2	46,000	20,694	45·0
5000—	4	24,000	5457	22·7
3000—	7	25,200	4805	19·1
2000—	3	6200	1300	21·0
1000—	4	5050	1242	24·6
500—	10	7224	1904	26·4
0—	7	2175	493	22·7

TABLE LXIV.

*Deaths from plague, September 1906—September 1907.*

(Grouped in 3-weekly periods.)

	Amritsar	Gujrat
—September 15th	1	—
—October 6th	4	—
—October 27th	5	18
—November 17th	35	97
—December 8th	132	59
—December 29th	346	173
—January 19th	389	201
—February 9th	639	677
—March 2nd	1109	1585
—March 23rd	1794	5766
—April 13th	3244	6417
—May 4th	5591	11,827
—May 25th	6867	15,823
—June 15th	2988	9357
—July 6th	707	2820
—July 27th	76	418
—August 17th	16	7
—September 7th	4	—
Total	23,947	55,245

*Amritsar 1905—1906.*

(4-weekly periods.)

—September 23rd	...	...	1
—October 21st	...	...	0
—November 18th	...	...	5
—December 16th	...	...	26
—January 13th	...	...	30
—February 10th	...	...	60
—March 10th	...	...	143
—April 7th	...	...	437
—May 5th	...	...	1896
—June 2nd	...	...	3010
—June 30th	...	...	431
—July 28th	...	...	20
—August 25th	...	...	2
Total			6061

APPENDIX IV.

*Further Note on the rates of mortality in villages of different sizes.*

On p. 81 I attempted to show that the difference in the rate of mortality, observed in most epidemics when one passed from large to small infected villages, could hardly be accounted for by assuming that the chance of infection reaching a colony of rats was independent and that the ratio between the number of human deaths and the number of plague-infected colonies was constant. I will here discuss the results which flow from the assumption that a single colony has a greater chance of being infected if it is situated in a large village than if it is in a small one. In other words I will assume that the chance of infection is not constant from group to group though constant within each group.

Let  $p_1$  be the chance of a colony becoming infected among the colonies situated in villages which contain on the average  $m_1$  colonies a piece. Similarly let  $p_2$  and  $m_2$  be corresponding values for another group of villages. Let  $p_1 + q_1 = 1$  and  $p_2 + q_2 = 1$ .

Then the ratio of the mortalities among infected villages of the two groups is<sup>1</sup>

$$\frac{1 - q_1}{1 - q_2} \times \frac{1 - q_2^{m_2}}{1 - q_1^{m_1}} \dots\dots\dots(1).$$

Since  $q_1$  and  $q_2$  are by definition less than unity this approaches

$$\frac{1 - q_1}{1 - q_2} \dots\dots\dots(2)$$

when  $m_1$  and  $m_2$  are both large. If  $q_1$  and  $q_2$  are fixed, (1) will be as small as possible when  $m_2$  is unity and  $m_1$  very large, in which case it approaches  $1 - q_1 = p_1$ .

The value of (1) lies accordingly between  $p_1$  and  $\frac{p_1}{p_2}$ , assuming that  $m_2$  cannot be greater than  $m_1$ .

But we have no means of determining the values of  $p_1$ ,  $p_2$ ,  $m_1$  and  $m_2$ . We may presume that  $p_1$  and  $p_2$  are proportional to the ratio:  $\frac{\text{Infected Villages}}{\text{Total Villages}}$  for each class and that  $m_1$  is to  $m_2$  in the ratio of the average populations of each class, but we cannot determine the absolute values<sup>1</sup>.

Simply as an arithmetical illustration, let us suppose that in the two groups of villages having populations 1000—3000 and 0—1000, the  $p$ 's are absolutely the ratios of infected to total villages, that  $m_2$  the

<sup>1</sup> Assuming that the average populations are  $m_1 n$  and  $m_2 n$  respectively where  $n$  is a constant.

number of colonies per village in the group 0—1000 is 1, and  $m_1 = 2$  or  $= 3$ . Then, taking Amritsar 1907,

we have  $q_1 = \frac{4.5}{2.46} = .183$  and  $q_2 = \frac{40.9}{78.2} = .523$ .

The ratio of mortality rate in infected villages 1000—3000 to mortality rate in infected villages 0—1000 will be .822 if  $m_1 = 3$  and  $m_2 = 1$ , .85 if  $m_1 = 2$  and  $m_2 = 1$ . The observed ratio is .63. The limits within which the ratio might be anticipated to fall are .817 and 1.71.

In this way, I have deduced the entries in Table LXVII from the data of Table LXV. In two cases, Amritsar 1905 and 1907, the observed ratio is smaller than the least value deduced from the above hypothesis. In the seven other illustrations the real value is between the theoretical limits, but no plausible values for  $p_1$ ,  $p_2$ ,  $m_1$  and  $m_2$  are suggested by the results. The general conclusion appears to be that, even as modified here, the hypothesis will not explain the facts.

TABLE LXV.

*Comparison of mortality for villages actually infected with that of whole population at risk.*

AMRITSAR.							
<i>Population 0—1000.</i>							
	Population of infected villages	No. of villages	No. of deaths	D.R. %	Population of all villages, in group	No. of villages	D.R. %
1902	13,151	20	298	2.27	357,961	782	.08
1903	163,074	281	10,286	6.31	357,961	782	2.87
1904	137,851	247	5177	3.76	357,961	782	1.45
1905	228,256	419	12,632	5.53	357,961	782	3.53
1906	77,707	142	2015	2.59	357,961	782	.56
1907	205,246	373	10,560	5.15	357,961	782	2.95
<i>Population 1000—3000.</i>							
1902	49,489	31	1008	2.04	378,657	246	.27
1903	275,965	175	16,163	5.86	378,657	246	4.27
1904	266,025	168	11,357	4.27	378,657	246	3.00
1905	336,362	215	13,554	4.03	378,657	246	3.58
1906	167,467	109	2785	1.66	378,657	246	.74
1907	318,149	201	10,370	3.26	378,657	246	2.74
ROHTAK.							
<i>Population 0—1000.</i>							
1904	12,604	23	376	2.98	137,080	299	.27
1905	65,434	120	4281	6.54	137,080	299	3.12
1906	911	2	9	.99	137,080	299	.01
1907	54,446	91	3224	5.92	137,080	299	2.35
<i>Population 1000—3000.</i>							
1904	27,432	17	619	2.26	251,662	152	.25
1905	197,942	119	11,751	5.94	251,662	152	4.67
1906	23,200	13	748	3.22	251,662	152	.30
1907	192,660	112	13,487	7.00	251,662	152	5.36

TABLE LXVI.

*Comparison of mortality for villages actually infected with that of whole population at risk.*

MOZAFFARNAGAR.

*Population 0—3000.*

	Population of infected villages	No. of villages	No. of deaths	D.R. %	Population of all villages, in group	No. of villages	D.R. %
1902	2137	1	3	·14	645,492	924	·00
1903	23,435	18	525	2·24	645,492	924	·08
1904	126,228	108	3115	2·47	645,492	924	·48
1905	298,663	273	9285	3·11	645,492	924	1·44
1906	70,485	54	1551	2·20	645,492	924	·24
1907	489,201	541	33,544	6·86	645,492	924	5·20

*Population over 3000.*

1902	23,444	1	1	·00	274,710	52	·00
1903	50,509	5	257	·51	274,710	52	·09
1904	131,227	20	2371	1·81	274,710	52	·86
1905	247,026	43	6961	2·82	274,710	52	2·53
1906	103,924	14	1526	1·47	274,710	52	·56
1907	264,202	49	16,365	6·19	274,710	52	5·96

HOSHIARPUR.

*Population 0—3000.*

1898	11,635	15	352	3·03	952,181	2085	·04
1899	3701	4	9	·24	952,181	2085	·00
1900	9636	11	50	·52	952,181	2085	·01
1901	33,760	43	332	·98	952,181	2085	·03
1902	340,675	464	10,824	3·18	952,181	2085	1·14
1903	No data :	—	—	—	952,181	2085	—
1904	506,168	844	24,642	4·87	952,181	2085	2·59
1905	555,936	940	18,079	3·25	952,181	2085	1·90
1906	176,690	284	3513	1·99	952,181	2085	·37
1907	530,305	879	14,745	2·78	952,181	2085	1·55

*Population over 3000.*

1898	5803	1	87	1·50	83,633	16	·10
1899	—	—	—	—	83,633	16	—
1900	—	—	—	—	83,633	16	—
1901	9030	2	112	1·24	83,633	16	·13
1902	63,755	12	864	1·36	83,633	16	1·03
1903	No data :	—	—	—	83,633	16	—
1904	65,952	12	1425	2·16	83,633	16	1·70
1905	63,305	12	1052	1·66	83,633	16	1·26
1906	52,038	9	612	1·18	83,633	16	·73
1907	68,079	13	962	1·41	83,633	16	1·15

TABLE LXVII.

*Ratio of the rate of mortality in infected villages 1000—3000,  
to the rate in infected villages 0—1000.*

District	Year	(1) Observed ratio	(2) Smallest theoretical	(3) Largest theoretical	(4) Theoretical for $m_1=3, m_2=1$	(5) Theoretical for $m_1=2, m_2=1$
Amritsar	1902	·90	·13	4·93	·38	·53
„	1903	·93	·71	1·98	·73	·78
„	1904	1·13	·68	2·16	·71	·76
„	1905	·73	·87	1·63	·88	·89
„	1906	·64	·44	2·44	·54	·64
„	1907	·63	·82	1·71	·82	·85
Rohtak	1904	·76	·11	1·45	·37	·53
„	1905	·91	·78	1·95	·79	·82
„	1907	1·18	·74	2·42	·75	·79



# XLVI. OBSERVATIONS ON PLAGUE IN EASTERN BENGAL AND ASSAM.

With Map and 5 Charts in Text and Plates II—XIV.

## CONTENTS.

	PAGE
A. Introduction . . . . .	158
B. History of plague in the province . . . . .	158
C. Method adopted for the study of the conditions present in the province . . . . .	162
(1) Observations made in Dacca . . . . .	162
(2) Observations made in the surrounding district . . . . .	163
D. Facilities for the importation of infection and for the spread of infection within the province . . . . .	169
(1) Physical features of the country . . . . .	169
(2) Propinquity to infected places . . . . .	169
(3) Communication with infected centres by rail, road, and water . . . . .	170
(4) Extent and nature of trade especially with plague centres . . . . .	171
E. Climate of Eastern Bengal and Assam . . . . .	172
F. An examination of the rats and their fleas found in houses . . . . .	173
(I) Data collected in Dacca . . . . .	173
(II) Data collected in the district . . . . .	183
G. Deductions to be drawn from the above data . . . . .	187
(1) The freedom of Eastern Bengal from plague is due mainly to the scarcity of rats in the houses of the people . . . . .	187
(2) The physical features of the country assist in keeping the province free from plague . . . . .	188
H. The causes for the comparative scarcity of rats in the houses . . . . .	189
(1) Habits of the people . . . . .	189
(2) Structure of their houses . . . . .	190
I. General conclusions . . . . .	192

## A. INTRODUCTION

THE Sanitary Commissioner with the Government of Eastern Bengal and Assam addressed the following letter to the Sanitary Commissioner with the Government of India :

"It has been suggested that an addition to our knowledge of plague might be gained by a study of the conditions under which the people of this Province are living seeing that they have not hitherto suffered from the disease in an indigenous form, and I have the honour to ask whether the members of the Plague Commission could make it convenient to extend their researches in this direction. I may add that the Local Government would be glad to welcome them and to afford them assistance in their work."

In compliance with this invitation the Advisory Committee arranged that the Commission working in India should make an inquiry in the Province of Eastern Bengal and Assam. This inquiry was started in December, 1909, and the results are contained in the following pages.

## B. HISTORY OF PLAGUE IN THE PROVINCE.

Since the commencement of the present outbreak of plague in India in 1896, the province of Eastern Bengal and Assam has suffered only to a very limited extent from the disease. With the exception of a small but definite epidemic of bubonic plague at Dibrugarh in the extreme north-east of the province, and a few doubtful cases occurring in a remote part of Manipur, the few epidemics that have occurred in the province have all been of pneumonic type. Some isolated cases of bubonic plague in addition have also been reported but a history of importation from outside the province could in each case be obtained. No evidence of a rat mortality except at Dibrugarh was ever recorded.

The following is a brief summary of the information obtained from the reports of various medical officers and government officials on the occurrence of plague in the province since the present outbreak of plague began in India in the end of the year 1896.

1. Backergunge, September, 1898. In September, 1898, 11 cases of pneumonic plague occurred at Shidakati and Abbynil, in the Nalchiti rural circle of Backergunge. The infection was said to have been imported from Calcutta; all persons attacked died. This epidemic was

minutely studied by the Royal Commission and is reported in detail in Vol. v. p. 691 of their report.

2. Pania villages (Dacca district), February–March, 1899. The next outbreak occurred at Pania villages in the Mounshiganj subdivision of Dacca. The epidemic originated in a case which was imported from Calcutta. The patient died in one of the villages on the day following his arrival from Calcutta. This case gave rise by direct contact or communication to 42 cases living in nine homesteads. Of the persons attacked 38 died. The disease was apparently of the pneumonic type. There was no history of rat mortality, and a note was made of the absence of any glandular swellings. No bacteriological examination of the sputum was undertaken.

3. Tippera, March, 1899. Six cases of plague were imported from Calcutta into various places in the Tippera district in March, 1899. Most of them were of the bubonic type and were not associated with cases of local origin.

4. Faridpur, March, 1899. A small outbreak of pneumonic plague occurred in two villages, viz. Bajitpur and Shirakhara in Madaripur subdivision of Faridpur district. There were in all 30 cases and 29 deaths. The source of the infection was not recorded. There were apparently no deaths amongst rats but two cases in Bajitpur were said to be of the bubonic type.

5. Dibrugarh (Lakhimpur district), May, 1903. An outbreak occurred in Dibrugarh in May, 1903. A man, a Dacca Mahomedan, was taken ill on 17th May with high fever and buboes. He died two days later. A large number of rats had been observed to be dying for some days previously and the rats first found were within 50 yards of his house; cultivations of the organisms obtained from the rat revealed a *cocco-bacillus* similar to plague. There was strong evidence connecting the disease with rats and grain golas (receptacles for storing grain). There were in all 36 cases and 22 deaths. Of these cases 23 were traceable directly, and four indirectly, to grain shops and golas. The epidemic was limited to foreigners, chiefly Marwaris who deal in grain. Most of the dead rats were found in Kayapati where also 20 out of the 36 cases lived. The cases seem to have been of the bubonic type but one case presented pneumonic symptoms.

6. Serajganj (Pabna district), March–April, 1906. A case was imported into Raghurbari village from a jute mill near Calcutta in March, 1906, and gave rise to 27 attacks and 24 deaths; all were of the pneumonic type though one was noted as having enlarged glands of

the neck. All were apparently infected by direct contact. There was no rat mortality observed.

7. Mymensingh, June–August, 1906. There were two outbreaks in Bajitpur village. Both outbreaks derived their infection from Calcutta and both were of the pneumonic type with a marked absence of buboes. In the first (June, 1906) outbreak five cases occurred and all of these died. In the second there were 50 cases and 45 deaths.

8. English Bazaar (Malda District), January, 1907. Infection was derived from Ballia in the United Provinces. Four persons infected by direct contact were attacked with plague and all died; no mention was made of the type of the disease but symptoms pointed to pneumonia.

9. Goalundo (Faridpur District), April, 1907. One imported case arrived in a steamer from Dighaghat suffering at the time from symptoms of bubonic plague.

10. Tippera, April, 1907. A case of pneumonic plague imported from Calcutta occurred at Kalikacha.

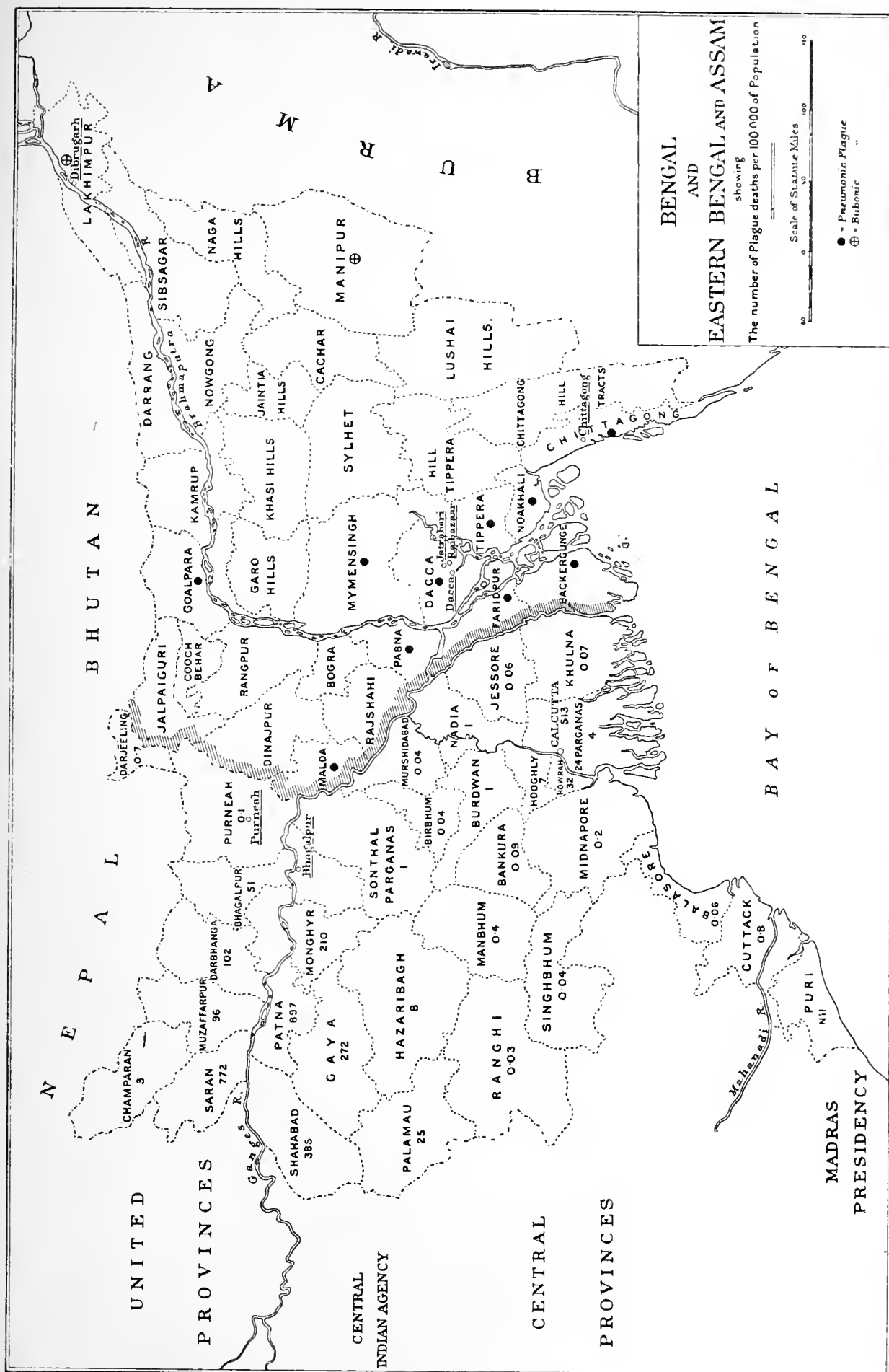
11. Manipur, June, 1907. About eight doubtful cases of bubonic plague with four deaths are said to have occurred near Imphal in Manipur district, a very wild, isolated and hilly country near the Burmese frontier. In the report it was hinted that the infection was probably derived from Burmah.

12. Goalpara, March, 1909. A plague case was imported into Goalpara from Calcutta in March, 1909. There was no mention of the type of the disease but no further cases were reported.

13. Chittagong, January, 1910. A bubonic plague case occurred at Chittagong in January, 1910, imported from the United Provinces.

14. Noakhali, June, 1910. A pneumonic plague outbreak occurred at Noakhali in June, 1910. The infection was imported from Calcutta. Fifty-one cases were reported of whom 45 died. The epidemic lasted one month and spread by direct contact. All the cases were of the pneumonic type of the disease and there was no mortality noted amongst rats.

The history of plague in this province thus shows that while a number of pneumonic cases of plague, imported into the province, gave rise to small epidemics of the disease, only a very few cases of bubonic plague were recorded. We have reason to believe, however, that the preponderance of imported pneumonic cases is only apparent, that in fact the pneumonic cases came to the notice of the authorities mainly because of the epidemics associated with them, while many cases of bubonic plague were imported into the province but were not recorded,



because they were not connected with epidemics. This is the more probable when we remember that only about 3 % of the cases of plague in India are of the pneumonic type, that no very elaborate precautions are taken to register all cases of plague in the province, and that communication with infected areas is freely allowed. We may conclude therefore that plague infection has been frequently brought into the province and that there are definite reasons for the disease not becoming endemic.

C. METHODS ADOPTED FOR STUDYING THE CONDITIONS PREVAILING IN THE PROVINCE.

(1) *Observations made in Dacca.*

Dacca is a town of about 90,000 inhabitants situated in a direct line only 160 miles from Calcutta where epidemics of plague are of annual occurrence. Daily communication is maintained with Calcutta so that the opportunities for the importation of infection are considerable. The town is the largest in the province; the houses are placed more closely together and the population concentrated on a smaller area than in the majority of places in the province. It is reasonable to believe therefore that, provided infection were once implanted in the town, the disease would make more headway among the rat population than in the more scattered villages of the district, and moreover the presence of the disease would readily come to the notice of the authorities more particularly because the supervision of the health of the town is in the hands of a qualified Medical Officer. We may assume then that opportunities for the importation of infection into Dacca have been considerable but that for some reason the disease has never succeeded in gaining a foothold in the town, so that whatever conditions are unfavourable to the prevalence of plague in the province may be presumed to be present to a marked degree in Dacca itself.

An additional reason for the selection of Dacca is to be found in the fact that owing to the size of the town a considerable number of rats could be caught daily without materially interfering with the rats and fleas infesting the place.

(a) *Work in the city.* The routine methods of rat and flea infestation carried out by the Commission in various places in India have already been described so frequently that it is scarcely necessary to repeat them here in detail. Observations on the usual lines were

commenced in the town in December, 1909, and continued daily throughout the course of the year. In order that the observations here might be compared with those made by the Commission in Poona and elsewhere in India, the methods adopted in conducting our inquiry were similar in all essential details to those employed in our previous work. A certain number of traps were set daily, simultaneously in three sections, in the city. The rats caught in the traps were brought in to the laboratory every morning for examination, the traps as usual being enclosed in flea-proof canvas bags.

(b) *In the laboratory and office.* The rats contained in the traps were examined in the usual way and all information thus obtained was collected into registers and summarised from time to time. Further, with the object of learning whether the structure of a house has any influence on the number of rats found in it, the houses in the city were classified in groups according to the structure of the wall, the character of the roof, the nature of the floor or plinth, and the number of rats caught in traps set in the houses so classified was noted. A certain number of traps<sup>1</sup> were also baited with different kinds of bait such as coconut, dried fish, plaintain etc., and these were set at random amongst those distributed in the ordinary routine; the results thus obtained were compared with the ordinary bread and oil bait, used throughout our observations.

(2) *Work in the surrounding district.*

(a) *Work in the non-plague area of Eastern Bengal and Assam.* A number of towns and villages were selected, as far as possible representative of every type in the province; these were visited and observations were made on the structure of the houses, the habits of the people, the number of rats found in the houses and the flea infestation of the rats, in order to compare the conditions found in them with those found in Dacca. The selected places were: Chittagong (a sea-port town in the extreme south-east), Dibrugarh (a large town in the extreme north-east), Jatrabari (a small village east of Dacca), and Raibazaar (a large village near Dacca).

The visits to these places were begun early in February when it became possible to leave Dacca, the work there having been so organised that it could be carried on efficiently during our temporary absence.

<sup>1</sup> The rats caught in these special traps were excluded in calculating the number of rats per 100 traps set throughout the year.

Each place was visited for a period of a week or ten days according to its size. A short period was selected to enable us to visit a number of places. This plan we considered to be better than devoting a longer period to fewer places for we were always able to compare the results obtained during our short visits (although the figures were small) with the prolonged observations in Dacca. We took with us on each visit from 40 to 70 traps according to the size of the place to be visited. The town or village having been divided up into a number of sections, batches of from eight to ten traps were allotted to each section so that even in so short a period as ten days a fair sample of the rats and their fleas for that particular time of the year was obtained.

(b) *The work in the neighbouring plague area of Bengal.* A reference to the map and Table I will show that, with the exception of Calcutta and two districts immediately adjoining it, practically all the plague has been confined to a comparatively small area comprising some eight to ten districts in the north-western portion of the province of Bengal. This latter area is roughly triangular in shape, with its base abutting on the United Provinces while its apex lies in the Bhagalpur district. A further examination of the distribution of the disease in this area shows that its greatest intensity is in the immediate neighbourhood of the Ganges river. Thus, while the district of Saran has an average annual plague death rate for the seven year period above mentioned of 77 per 10,000 of the population, the adjoining district of Champaran has only a plague death rate of 0·3 per 10,000 for the same period. Again in the Bhagalpur district practically all the plague occurs in the two or three "thanas" or subdivisions which are nearest the river, that is, in the centre of the district.

The monthly distribution of plague deaths in Bengal and Calcutta city is shown separately in Tables II and III for the years 1901 to 1909 in the case of Bengal, and for 1900 to 1909 in the case of Calcutta. Charts I and II graphically demonstrate the seasonal prevalence of plague in Bengal and Calcutta respectively.

Of the various towns in the triangular plague area of Bengal, the largest one, nearest to the border of Eastern Bengal, which had been visited by plague epidemics in several previous years, was Bhagalpur, the capital town of the Bhagalpur district in Behar. We therefore determined, after our visit to Chittagong, to proceed to Bhagalpur, to compare the conditions found there with those observed in Eastern Bengal. Bhagalpur had the advantage too that at the time of our visit plague was absent from the town nor had the disease been prevalent



for the past two years. We obtained results therefore, as far as rat and flea infestation was concerned, which more nearly approximated to the normal than had the town recently suffered from the disease.

TABLE I.

*Showing the total and the mean plague death rate per mille, in the different districts of the Province of Bengal (except Calcutta city) for seven years from 1903 to 1909 (inclusive).*

Names of districts	Population according to census of 1901	Total plague deaths from 1903 to 1909 (inclusive)	Total plague death-rate per mille from 1903 to 1909 (inclusive)	Mean plague death-rate per mille from 1903 to 1909 (inclusive), 7 years
Burdwan	1,532,475	144	0·094	0·013
Birbhum	902,280	3	0·003	0·0004
Bankura	1,116,411	7	0·006	0·0009
Midnapore	2,789,114	33	0·012	0·002
Hooghly	1,049,282	520	0·496	0·071
Howrah	850,514	1882	2·213	0·316
24 Parganas	2,078,359	544	0·262	0·037
Nadia	1,667,491	112	0·067	0·010
Murshidabad	1,333,184	4	0·003	0·0004
Jessore	1,813,155	8	0·004	0·0006
Khulna	1,253,043	7	0·005	0·0007
Patna	1,624,985	102,022	62·783	8·969
Gaya	2,059,933	39,166	19·013	2·716
Shahabad	1,962,696	52,866	26·935	3·848
Saran	2,409,509	130,161	54·020	7·717
Champaran	1,790,463	414	0·231	0·033
Muzaffarpur	2,754,790	18,574	6·742	0·963
Darbhanga	2,912,611	20,806	7·143	1·020
Monghyr	2,068,804	30,392	14·691	2·099
Bhagalpur	2,088,953	7514	3·597	0·514
Purneah	1,874,794	16	0·008	0·001
Darjeeling	249,117	12	0·048	0·007
Sonthal Parganas	1,809,737	131	0·072	0·010
Cuttack	2,062,758	116	0·056	0·008
Balasore	1,071,197	4	0·004	0·0006
Puri	1,017,284	Nil	Nil	Nil
Hazaribagh	1,177,961	676	0·574	0·082
Ranghi	1,187,925	2	0·002	0·0003
Palaman	619,600	1096	1·769	0·253
Manbhum	1,301,364	37	0·028	0·004
Suighbhum	613,579	4	0·003	0·0004

30,346 deaths from plague took place in Calcutta city from 1903 to 1909 (inclusive). Total plague death rate per mille for the period is 35·794 and the mean plague death rate is 5·11.

TABLE II.

Showing the monthly figures of plague deaths in the districts of Bengal, including towns, from 1901 to 1909 (both years inclusive). The plague deaths of Calcutta city have been excluded.

	1901	1902	1903	1904	1905	1906	1907	1908	1909	Total plague deaths, past 9 years (1901 to 1909), both years inclusive	Average monthly plague deaths, past 9 years (1901 to 1909), both years inclusive
January	9034	2725	9518	8320	20,682	5316	5426	1522	799	63,342	7038·0
February	14,131	3821	11,750	13,851	22,472	10,212	10,847	3267	1056	91,407	10,156·3
March	26,976	5756	17,449	21,958	38,037	19,197	23,524	5593	1773	160,263	17,807·0
April	15,651	3536	9387	8881	23,418	15,561	29,420	2565	702	109,121	12,124·6
May	2120	1053	952	1375	7825	1451	7171	181	300	22,428	2492·0
June	140	135	108	536	1352	189	696	26	77	3259	362·1
July	18	40	62	480	92	60	245	11	55	1063	118·1
August	47	20	50	1785	198	386	424	16	124	3050	338·9
September	271	92	205	1659	340	533	453	66	180	3799	422·1
October	350	544	541	1011	485	476	279	126	230	4042	449·1
November	627	1749	2151	2166	1169	851	393	287	903	10,296	1144·0
December	1381	6218	5285	8725	2642	2781	1133	509	3026	31,700	3522·2
Yearly total	70,746	25,689	57,458	70,747	118,712	57,013	80,011	14,169	9225	503,770	55,974·4

TABLE III.

Showing the monthly plague deaths in Calcutta city from 1900 to 1909 (both years inclusive).  
Figures obtained from the reports of Sanitary Commissioner.

Months	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	Total plague deaths, past ten years (1900 to 1909), both years inclusive	Average monthly plague deaths, past 10 years (1900 to 1909), both years inclusive
January	218	283	169	260	73	186	103	60	58	64	1474	147.4
February	653	703	506	1058	290	408	124	89	97	84	4012	401.2
March	3531	3944	2282	3140	1170	1503	684	454	341	294	17,343	1734.3
April	1855	2011	2457	2801	2293	3509	935	1420	501	386	18,168	1816.8
May	672	416	1266	499	529	1093	306	972	364	717	6834	683.4
June	451	174	248	71	159	198	190	283	130	273	2177	217.7
July	230	58	78	47	44	58	36	63	91	138	843	84.3
August	229	91	69	56	19	48	44	26	67	53	702	70.2
September	229	60	45	74	12	70	38	30	32	28	618	61.8
October	90	66	35	83	12	24	37	30	40	36	453	45.3
November	9	107	45	78	31	74	47	82	28	21	522	52.2
December	108	97	78	57	71	101	63	80	32	23	710	71.0
Yearly total	8275	8010	7278	8224	4703	7272	2607	3589	1781	2117	53,856	5385.6

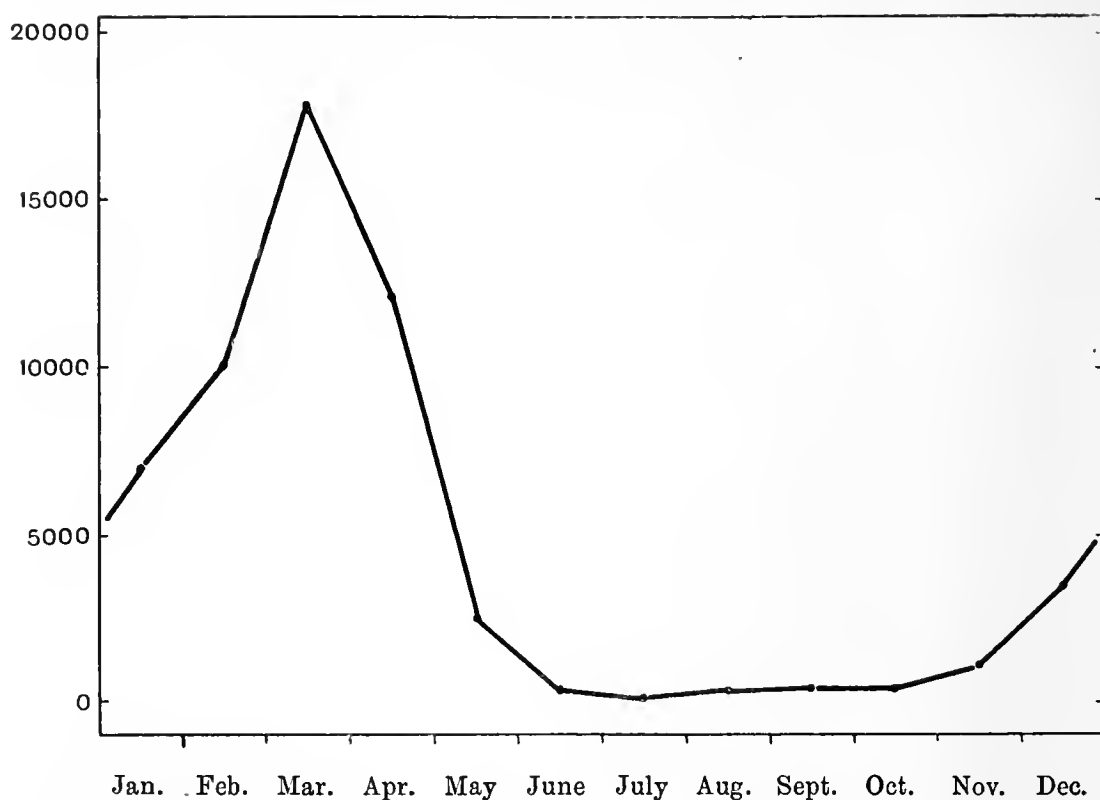


Chart I. Seasonal prevalence of human plague in Bengal exclusive of Calcutta.

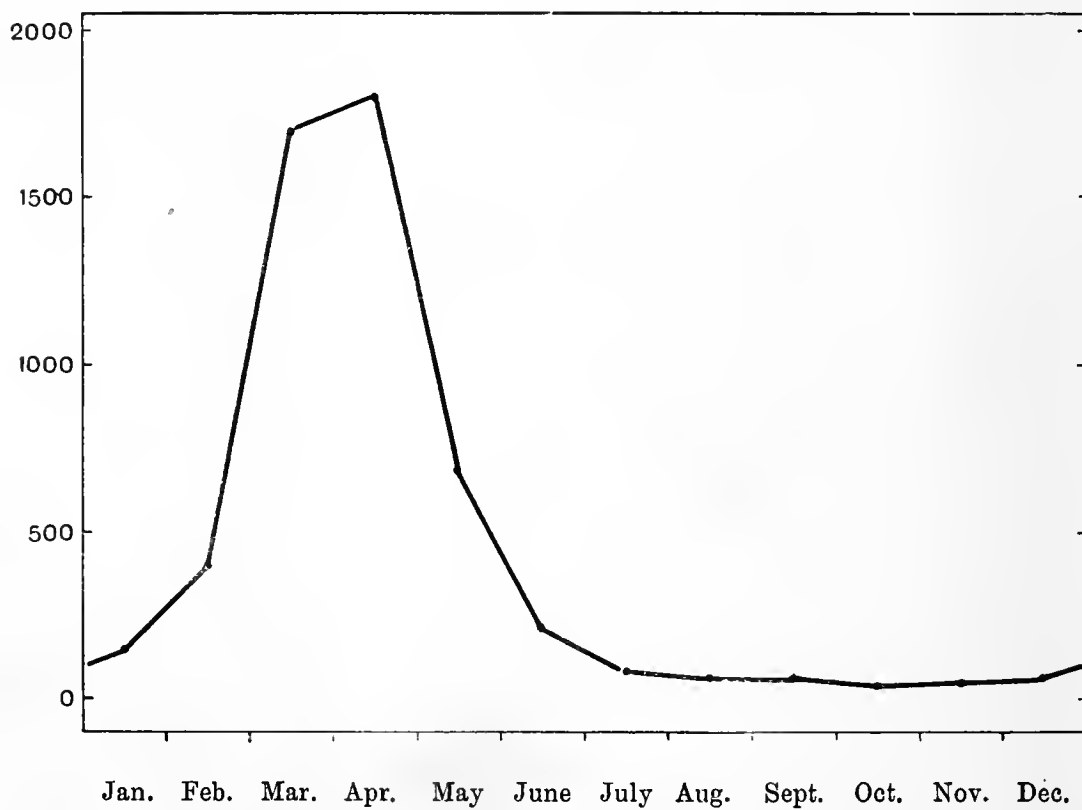


Chart II. Seasonal prevalence of plague in Calcutta.

When the work in Bhagalpur had been completed we found that the conditions there differed so remarkably from those observed in Eastern Bengal that we determined to examine another town. We decided to select one which had been free from plague but which was situated between the boundary of Eastern Bengal and Bhagalpur; our choice fell on Purneah.

D. FACILITIES FOR THE IMPORTATION OF INFECTION AND FOR THE SPREAD OF INFECTION FROM ONE PLACE TO ANOTHER WITHIN THE PROVINCE.

(1) *Physical features of the country.*

The province of Eastern Bengal and Assam has an area of 111,569 square miles, and in shape is roughly quadrangular (see map).

On the north it is bounded by the Himalayan mountains. On the east it is separated from Burmah by an almost equally inaccessible series of mountains covered with dense forests, and on the south it is limited by the Bay of Bengal. The western portion of the province adjoins Bengal proper, but is separated from Bengal along the greater part of this western boundary by the Ganges river.

Projecting to the plain from the hilly eastern border is the range of the Khasi and Garo hills covered with dense jungle. These hills almost completely divide Assam in the north from Eastern Bengal in the south. With the exception of these hills and certain other hilly tracts the major part of the province consists of the rich fertile and low-lying alluvial valleys of the Brahmaputra and Ganges rivers, with their many tributaries.

It will thus be seen that impenetrable mountain ranges practically limit the importation of infection from the north and east while the sea on the south and the Ganges river on the west greatly curtail the opportunities for the importation of infection in these directions.

(2) *Propinquity to infected places.*

We have already referred to the plague-infected areas in Bengal. We pointed out that one area existed in Calcutta and its neighbourhood while another was to be found in the north-western portion of that province. These plague-infected areas are both separated from the

province of Eastern Bengal and Assam by a considerable tract of relatively plague-free country. Thus the Calcutta area is separated from Eastern Bengal and Assam by the plague-free districts of Nadia, Jessore, Khulna, and 24 Paganas; while between the other plague area and Eastern Bengal the plague-free districts of Purneah and Sonthal Paganas intervene.

(3) *Communication with infected places by rail, road and water.*

There are two main systems of railway which carry traffic from plague-infected areas into Eastern Bengal and Assam. The Eastern Bengal State Railway passes from west to east along the northern portion of the province and carries traffic from the north-western plague-infected portion of Bengal into the province. The Assam-Bengal Railway carries traffic from the port of Chittagong in the south of the province in a northerly direction to Dibrugarh. This railway is the means by which a large trade with Calcutta is maintained. These two main railway systems are developing rapidly, but direct communication along them is frequently interrupted especially in the deltaic area of the Ganges and Brahmaputra. The numerous mouths of those large rivers with their shifting beds render bridge building practically impossible.

There are very few good roads in Eastern Bengal, especially in the southern parts of the province. This is probably owing to the frequent flooding of the low-lying country, the sandy nature of the soil, the scarcity of stone and the many intersecting streams which often change their course.

Navigable rivers and canals are numerous and afford the chief means of communication between one place and another within the province. Travelling by this means although cheap is yet very slow. Grain conveyed from Calcutta to, for instance, Dibrugarh would take several weeks to reach its destination by country boat and more than a week by steamer. It is important to bear this in mind in connection with the possible conveyance of infection by this channel. Owing to the melting of the winter snows in the Himalayas and the heavy rainfall during the six months May to October, the rivers and canals overflow and flood the low-lying country which, as we have seen, constitutes the major part of the province. Communication between village and village during this season is greatly interrupted. The floods

are generally so extensive as to prevent easy communication even between one tenement and another in the same village. Some photographs showing the flooding which takes place during the rainy season are here reproduced (figs. 1 and 2). In one of them a man may be seen making his way over the flooded fields in a large earthenware vessel, a common mode of progression during this season.

(4) *Extent and nature of the trade especially with plague centres.*

The population of the province in 1901 was 30,961,459. The density of the population, especially in the fertile villages, is very great and frequently lies between 950 to 500 per square mile; yet large towns and villages are few. Only 2% of the population were enumerated in the 61 towns of the province. The villages are generally made up of a number of hamlets scattered over the plain. These hamlets are situated on slight elevations and are surrounded on all sides by low-lying land which, as we have seen, for several months of the year is covered with water (see fig. 3).

Agriculture is the chief occupation of the people. The land is fertile and yields crops readily. Famines are unknown. The population is therefore generally well-to-do; extreme poverty such as is common in other parts of India is seldom met with. The staple food crop is rice which is produced in sufficient quantity to meet the requirements of the inhabitants; 60% of the total cropped area is covered with this cereal. In Assam, however, large areas are occupied by tea gardens. The labourers on these gardens come from many parts of India outside the province. A large and ever increasing cosmopolitan population is thus springing up in this part. The habits and food supply of these people differ from those of the indigenous inhabitants, and this is an important point to remember in connection with the importation of plague infection. One of the largest towns in this tea area is Dibrugarh in the extreme north-east of the province which, as we have seen, has passed through one mild epidemic of plague.

Very little grain is imported into the province since most of that which is consumed is grown locally. The chief imports are cotton piece goods, gunny bags (new), salt, sugar, kerosine oil, etc.—materials in fact which are little likely to carry plague infection in the form of infected rats or rat fleas.

## E. CLIMATE OF EASTERN BENGAL AND ASSAM.

It is difficult to give any general account of the climatic conditions in a province like Eastern Bengal and Assam, the hilly tracts differing so remarkably from the low valleys. Roughly, however, the province, from a climatological point of view, may be divided into Assam in the north and Eastern Bengal in the south.

(a) The mean *temperature* in the northern portion of the province seldom rises above 80° F. except in July, August and September and is generally below 75° F. for seven months in the year. In the southern portion of the province the mean temperature remains more or less above 80° F. from April till October. The diurnal variation of the temperature is small, being about twenty degrees during the winter months, fifteen degrees in April, May, June, October and November, while from July to September there is only a diurnal variation of about ten degrees.

(b) The relative *humidity* in Assam never falls below a monthly mean of 80% of saturation, but in Eastern Bengal considered as a whole the monthly mean humidity is below 80% from January to April.

(c) The *rainfall* in the northern portion of the province averages about one hundred inches in the year but the rainfall differs remarkably in the hills and plains. At Cherrapungi for example the annual rainfall averages four hundred and fifty-eight inches (nine hundred and five inches are said to have fallen in 1861) while at Lanka in the Kapli valley in Nowgong the average fall is less than forty-three inches. The greatest precipitation occurs in the months May to October, with an average of over ten inches during each of these months. In the southern portion of the province the average rainfall is about seventy-five inches, the greatest precipitation occurring from May to September.

The climate of Eastern Bengal and Assam is thus characterised by coolness and extreme humidity, the natural result of the great water surface over which evaporation takes place and the close proximity of hill ranges on which an excessive precipitation occurs. Its most distinguishing feature is the copious rainfall between March and May, a time when precipitation over the rest of India is at its minimum.

Table IV gives the normal temperature and humidity calculated from readings made at Narayanganj Observatory near Dacca town. The table also shows the calculated average mean monthly temperature and 8 a.m. humidity for the year of our observations, 1910.



TABLE IV.

Showing the normal monthly mean temperatures and 8 a.m. humidity as well as mean monthly temperatures and 8 a.m. humidity for 1910 at Narayanganj.

Month	Normal				1910			
	Mean max. temp.	Mean min. temp.	Mean of max. and min. temp.	Mean humidity at 8 a.m.	Mean max. temp.	Mean min. temp.	Mean of max. and min. temp.	Mean humidity at 8 a.m.
January	77·9°	55·2°	66·5°	87 %	75·4°	54·3°	64·9°	83 %
February	82·2	58·6	70·4	84	81·7	57·6	69·6	79
March	90·0	68·2	79·1	83	88·0	67·6	77·8	79
April	93·2	74·3	83·7	82	91·9	73·9	82·9	81
May	91·3	75·8	83·5	85	91·2	75·5	83·4	83
June	89·0	78·3	83·7	89	88·7	78·0	83·3	89
July	88·3	79·2	83·7	90	87·4	77·8	82·6	89
August	87·5	79·0	83·3	90	87·9	78·9	83·4	88
September	88·5	78·9	83·7	88	89·0	79·0	84·0	86
October	87·9	75·3	81·6	84	88·5	77·5	83·0	87
November	83·5	66·0	74·7	85	83·0	65·0	74·0	78
December	78·0	57·3	67·7	87	76·8	56·2	66·5	83

#### F. AN EXAMINATION OF THE RATS AND THEIR FLEAS FOUND IN HOUSES.

(I) Data collected in Dacca town from 13th December, 1909 to 31st December, 1910.

##### (a) Rats.

(1) If we include musk rats in the category of rats, then four species were found in Dacca houses. They were present in the following proportions:

Species	Number of rats	Proportion %
<i>M. rattus</i>	5704	48·5
<i>Gunomys varius</i>	2064	17·6
Musk rats	2337	19·9
Mice	1650	14·0

In all a total of 11,755 rats were caught and examined in Dacca town. Of these 5704 were *M. rattus* and among them 595 belonged to that variety with a white belly which we have occasionally referred to in our previous reports as *Mus rattus* var. *alexandrinus*.

It is of interest to compare the proportion of *M. rattus* to other rats caught in Dacca with similar figures obtained in Poona, Belgaum, Bombay and Madras, thus :

Place	<i>M. rattus</i> to all rats	Other kinds of rats
Dacca	48.5 %	Many <i>G. varius</i> , musk rats and mice found.
Poona	98.6	A few musk rats and mice.
Belgaum	95.7	A few musk rats, mice and bandicoots.
Bombay	66.2	Many <i>M. decumanus</i> , a few musk rats, mice and <i>G. varius</i> .
Madras	49.4	Many mice 46.1 %, a few musk rats and bandicoots.

The proportion of rats caught to traps set is shown month by month in Table V. In Chart III a contrast is drawn between the number of rats caught per month per 100 traps set in Dacca with similar figures obtained during the same period in Poona city. It is here necessary to state that, from the time (January, 1910) when the Poona record shown in the chart begins, plague had been absent from the city for one year and eight months. During this plague-free period in Poona the rats caught per 100 traps set had steadily risen from sixteen rats to approximately forty. It will be seen from the chart that the number of rats caught per 100 traps set still continued to rise during the year 1910, in the complete absence of plague, to approximately seventy-five

TABLE V.

Showing the number of *Mus rattus*, *Gunomys varius*, mice and musk rats per 100 traps set, in monthly periods. Dacca, 1909—1910.

Period	Traps set	Rats caught				Rats per 100 traps set			
		<i>Mus rattus</i>	<i>G. varius</i>	Mice	Musk rats	<i>Mus rattus</i>	<i>G. varius</i>	Mice	Musk rats
December 13th } to 31st, 1909 }	2945	274	63	240	87	9.3	2.1	8.1	3.0
January 1910	5334	391	95	281	149	7.3	1.8	5.3	2.8
February „	4953	431	60	113	142	8.7	1.2	2.3	1.1
March „	7491	490	170	80	105	6.5	2.3	1.1	1.4
April „	7153	348	154	77	139	4.9	2.2	1.1	2.4
May „	7225	422	131	78	177	5.8	1.8	1.1	2.4
June „	7436	483	200	93	153	6.5	2.7	1.3	2.1
July „	7151	433	129	66	242	6.1	1.8	0.9	3.4
August „	6068	370	219	52	169	6.1	3.6	0.9	2.8
September „	7074	486	169	44	165	6.9	2.4	0.6	2.3
October „	6143	468	212	115	182	7.6	3.5	1.9	3.0
November „	6792	621	246	162	303	9.1	3.6	2.4	4.5
December „	6723	487	216	222	317	7.2	3.2	3.3	4.7

rats for every 100 traps set. The chart clearly shows that very few rats were caught in Dacca in proportion to the number of traps set, and contrasts this experience with that observed by us in other parts of India, especially in such a plague-infected city as Poona.

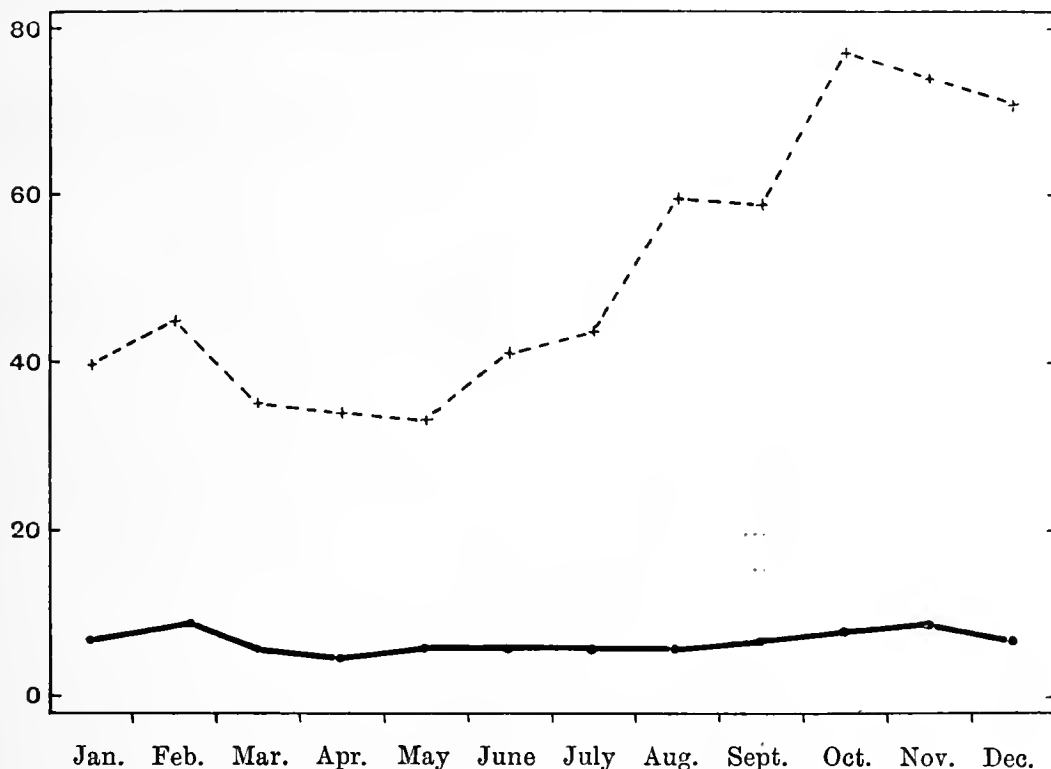


Chart III. *Rattus* caught per 100 traps in Dacca (●—●—●), and in Poona (+---+) in 1910.

(2) *Seasonal variation in the number of rats caught in Dacca.*

Figures bearing on this matter are to be seen in Table V where it will be noted that, except in the case of mice, there is not much seasonal variation in the number of rodents caught in the houses; there is however a tendency for all kinds of rats to be more readily caught in houses during the cold weather, an observation which accords with our experience in other parts of India where we have worked.

(3) *Breeding season of Mus rattus in Dacca.*

Statistics in this connection are shown in Tables VI and VII and these are graphically depicted in Chart IV. It is evident that although breeding takes place all the year round, the greatest number of adult females pregnant is found in the months of February, March, April and

May. This increase in the number of pregnant females is followed in the months of March, April, May, June and July by an increase in the number of young rats caught. The breeding season among rats here therefore is most active during the hot weather and this observation is in harmony with our experience in other parts of India (Vol. VII. pp. 749, 906; X. pp. 457, 520).

TABLE VI.

*Showing the percentage of adult female rattus pregnant in monthly periods. Dacca.*

Period	Adult female <i>rattus</i>	Adult females pregnant	Percentage of adult females pregnant
December 13th to 31st 1909	97	19	19·6
January 1910 ...	163	29	17·8
February „ ...	171	61	35·7
March „ ...	159	62	39·0
April „ ...	83	28	33·7
May „ ...	136	59	43·4
June „ ...	151	44	29·1
July „ ...	151	45	29·8
August „ ...	144	43	29·9
September „ ...	205	64	31·2
October „ ...	179	50	27·9
November „ ...	218	53	24·3
December „ ...	184	38	20·7

TABLE VII.

*Showing the percentage of young rattus in monthly periods. Dacca.*

Period	<i>Rattus</i> weighed	Young <i>rattus</i>	Percentage of young <i>rattus</i>
December 13th to 31st 1909	274	86	31·4
January 1910 ...	391	109	27·9
February „ ...	431	128	29·7
March „ ...	490	223	45·5
April „ ...	348	187	53·7
May „ ...	422	187	44·3
June „ ...	483	210	43·6
July „ ...	433	176	40·6
August „ ...	370	112	30·3
September „ ...	486	187	38·5
October „ ...	468	154	32·9
November „ ...	621	228	36·7
December „ ...	487	130	26·7

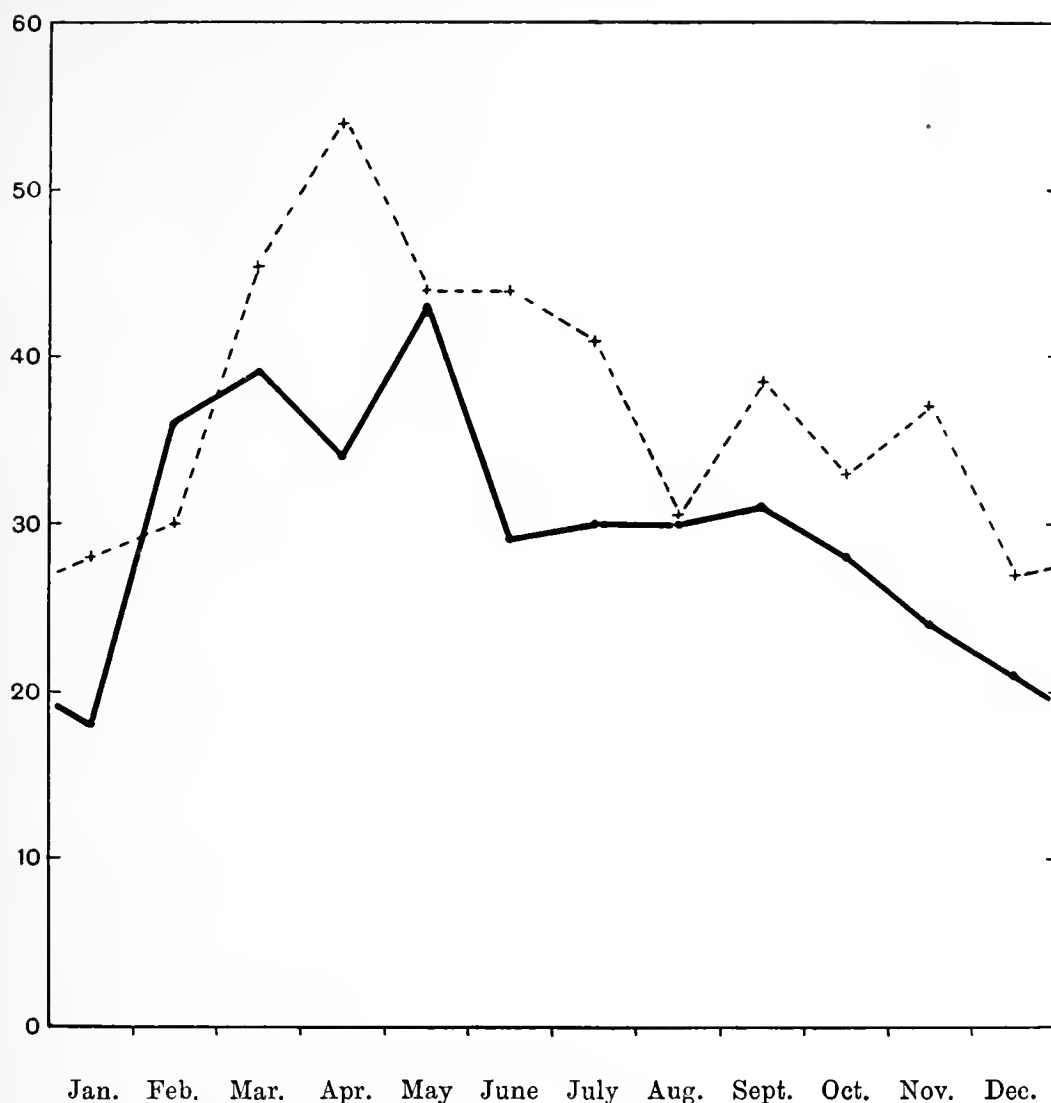


Chart IV. Percentage of female rats pregnant (—●—).  
Percentage of young rats (+----+).

(4) *Other observations bearing on the habits of rats.*

We have already mentioned that a certain number of traps were set with the object of ascertaining the comparative rat infestation of different types of houses and again that other traps were set with the object of testing the efficiency of different kinds of baits. The material collected in this way is displayed in Tables VIII to XII. A few remarks are here necessary to explain these tables. The words "pucca" and "kutchra," when applied to buildings, are commonly used in India to indicate on the one hand a well-made, solid brick and mortar or stone

building and on the other hand a flimsy cheap shelter of mud or bamboo or palm matting.

In Table VIII figures are given which show the result of trapping buildings according as they were regarded as "pucca houses or shops," "kutcha houses or shops," "godowns (store houses) or stables." This table shows that apart from godowns or stables more *M. rattus* were taken in well-built houses than in "kutcha" houses. It is interesting to note that this experience is exactly reversed in the case of musk rats and suggests the possibility that the one species is inimical to the other. Experiments have shown that the musk rat fiercely attacks and kills *M. rattus* when the two species are confined together in a cage. This is a matter of no little importance when we remember that the musk rat rarely if ever suffers from plague (Vol. VII. pp. 760, 904; X. p. 456).

TABLE VIII.

*Showing the number of Mus rattus, mice, musk rats and G. varius per 100 traps set, arranged according to the nature of house, shop or godown. Dacca.*

Three series : Jan. 9 to 30, 1910, June 20 to July 17, 1910, Oct. 10 to Nov. 6, 1910.

Nature of house, shop or godown	Traps set	Rats caught				Rats per 100 traps set			
		<i>Rattus</i>	Mice	Musk rats	<i>G. varius</i>	<i>Rattus</i>	Mice	Musk rats	<i>G. varius</i>
Pucca house or shops	9741	865	303	290	329	8·9	3·1	3·0	3·4
Kutcha „ „	7554	245	126	242	125	3·2	1·7	4·5	1·7
Others, including go- downs and stables	183	31	3	5	16	16·9	1·6	2·7	8·7

TABLE IX.

*Showing the number of Mus rattus, mice, musk rats and G. varius per 100 traps set in houses arranged according to the nature of the roof of the houses. Dacca.*

Three series combined : Jan. 31 to March 6, 1910, July 18 to Aug. 14, 1910, Nov. 7 to Dec. 4, 1910.

Nature of roof	Traps set	Rats caught				Rats per 100 traps set			
		<i>Rattus</i>	Mice	Musk rats	<i>G. varius</i>	<i>Rattus</i>	Mice	Musk rats	<i>G. varius</i>
Masonry roof	12,498	1046	224	437	357	8·4	1·8	3·5	2·9
Tin „	5358	325	126	195	66	6·1	2·4	3·6	1·2
Thatch „	1407	55	31	51	27	3·9	2·2	3·6	1·9
Other „	2	0	0	0	0	0	0	0	0

Table IX gives the result of trapping when regard is paid to the character of the roof of the buildings in which the traps are set. Here it appears that buildings with masonry roofs harboured the greatest proportion of rats, that the next largest proportion were caught in buildings with "tin" or corrugated iron roofs, and that buildings with roofs made of thatch or matting sheltered fewest rats. Tables X and XI give the results of trapping when regard is paid to the nature of the walls and floors of the buildings trapped.

TABLE X.

*Showing the number of Mus rattus, mice, musk rats and G. varius per 100 traps in houses arranged according to the nature of wall. Dacca.*

Four series: Dec. 5, 1909 to Jan. 31, 1910, March 7 to April 24, 1910, April 25 to May 22, 1910, August 15 to Sept. 11, 1910.

Nature of wall	Traps set	Rats caught				Rats per 100 traps set			
		<i>Rattus</i>	Mice	Musk rats	<i>G. varius</i>	<i>Rattus</i>	Mice	Musk rats	<i>G. varius</i>
Brick wall	19,279	1437	296	469	637	7.5	1.5	2.4	3.3
Mud	4619	183	49	83	27	4.0	1.1	1.8	0.6
Chattac ,,	3059	149	43	72	55	4.9	1.4	2.4	1.8
Other ,,	2297	48	29	74	40	2.1	1.3	3.2	1.7

TABLE XI.

*Showing the number of rats per 100 traps set, arranged according to the nature of the floor. Dacca.*

Two Series: May 29 to June 19, 1910, Sept. 12 to Oct. 9, 1910.

Nature of floor	Traps set	Rats caught				Rats per 100 traps set			
		<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>	<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
Pucca	7981	696	92	214	224	8.7	1.2	2.7	2.8
Kutchia	5173	222	44	106	96	4.3	0.8	2.0	1.8

These results, however, do not give a true indication of the efficiency of the particular materials used in the construction of the buildings in preventing the ingress of rats or of affording shelter or protection to them; other factors of greater importance, such as the situation of the buildings, the habits of the people dwelling in the houses, the size of the buildings<sup>1</sup>, the nature of the furniture and the other materials

<sup>1</sup> "Pucca" are generally considerably larger than "kutchia" houses.

stored in them, all play some, or even a greater part, in encouraging or discouraging the presence of rats in houses. A well-constructed house is not of itself a safeguard against rats although other conditions remaining constant a "pucca" house must of necessity be better than a "kutcha" one in this respect. Our figures simply serve to show once again that a well-constructed house in itself is not a complete protection against rat infestation.

Table XII gives the results of tests to ascertain which of a number of baits were most effective in attracting rats. It will be seen that bread and plantain are more efficient as baits for *Mus rattus* than cocoa-nut or dried fish.

TABLE XII.

*Showing the average number of Mus rattus, mice, musk rats and G. varius per 100 traps set according to the nature of bait.*

Kind of bait	Traps set	Rats caught				Rats per 100 traps set			
		<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>	<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
Plantain	2012	161	48	44	42	8.0	2.4	2.2	2.1
Bread	21,090	1527	457	709	485	7.2	2.2	3.4	2.3
Cocoa-nut	1851	93	34	53	28	5.0	1.8	2.8	1.5
Bread	19,736	1392	330	607	547	7.1	1.7	3.1	2.7
Dry fish	1833	61	27	47	68	3.3	1.5	2.6	3.7
Bread	18,973	1254	240	552	545	6.6	1.3	2.9	2.9

(5) *Pathological lesions met with in rats in Dacca.*

The only pathological lesions in rats that were of particular interest to us were lymphatic and splenic abscesses and signs of old perisplenitis, adhesions, and scars in the spleen. The presence of these conditions was carefully noted when met with in order to compare their frequency with similar conditions observed in Poona, Bombay and Belgaum where (Vol. X. p. 335) similar lesions among rats were attributed to epizootic plague. Our findings as regards Dacca are summarised in Table XIII. We may remark in connection with this table that almost all the lesions recorded could, to a trained eye, in the light of our subsequent experience, be distinguished from true resolving plague lesions. We do not intend here to enter into the differences between the lesions found in Dacca and those noted in Poona and Belgaum but merely record the fact that appearances may be met with in rats which have not been



exposed to plague which, to the unskilled observer, may be mistaken for resolving plague lesions. The former appear to exhibit some seasonal variation in frequency.

TABLE XIII.

*Showing the percentage of adhesions, scars, perisplenitis, and peripheral and visceral abscesses in Mus rattus for the year 1910 in quarterly periods. Dacca, Jan. 1st to Dec. 31st, 1910.*

Quarter ending	<i>M. rattus</i> weighed	Numbers of						Percentage				
		Adhesions	Scars	Perisplenitis	Peripheral abscesses	Visceral splenic abscesses		Adhesions	Scars	Perisplenitis	Peripheral abscesses	Visceral splenic abscesses
March 1910	1312	5	12	11	5	1		0·38	0·91	0·84	0·38	0·08
June „	1253	3	14	4	2	1		0·24	1·12	0·32	0·16	0·08
September „	1289	2	13	5	2	1		0·16	1·01	0·39	0·16	0·08
December „	1576	3	17	5	1	Nil		0·19	1·08	0·32	0·06	Nil

TABLE XIV.

*Showing the average number of fleas per G. varius, mice, musk rats, and Mus rattus in monthly periods. Dacca, 1909—1910.*

Monthly periods	<i>G. varius</i>			Mice			Musk rats			<i>M. rattus</i>		
	Flea counts made	Total fleas	Average fleas	Flea counts made	Total fleas	Average fleas	Flea counts made	Total fleas	Average fleas	Flea counts made	Total fleas	Average fleas
Dec. 13th to 31st, 1909	59	45	0·8	231	28	0·1	77	47	0·6	257	660	2·5
January 1910	93	167	0·8	271	69	0·3	70	58	0·8	388	1235	3·2
February „	57	209	3·7	96	19	0·2	28	15	0·5	421	2088	5·0
March „	166	732	4·4	71	25	0·4	23	8	0·3	464	2576	5·6
April „	150	695	4·6	70	33	0·5	19	46	2·4	339	2777	8·2
May „	118	599	5·1	71	45	0·6	30	23	0·8	402	2861	7·1
June „	195	1306	6·7	90	34	0·4	23	10	0·4	445	2655	6·0
July „	118	731	6·2	65	26	0·4	50	50	1·0	415	1929	4·6
August „	207	783	3·8	49	19	0·4	39	27	0·7	355	1213	3·4
September „	164	331	2·0	39	19	0·5	30	14	0·5	466	1092	2·3
October „	189	264	1·4	95	33	0·3	43	25	0·6	430	740	1·7
November „	203	604	3·0	135	44	0·3	37	20	0·5	573	1667	2·9
December „	183	454	2·5	188	27	0·1	31	6	0·2	442	1210	2·7
Total	1902	6920	3·6	1471	421	0·3	500	349	0·7	5397	22,703	4·2

## (b) Rat fleas.

(1) With the exception of a few human and cat fleas caught on rats in Dacca town, all the fleas found were *X. cheopis*. Of the four varieties of rats (including for the sake of convenience the musk rat) found in Dacca only two, viz. *M. rattus* and *G. varius*, harboured this flea in any numbers. The mouse and musk rat carried very few fleas. Table XIV gives the average number found on each species of rat in monthly periods.

(2) Considering only the fleas found on *M. rattus* a very distinct seasonal prevalence was observed. This seasonal variation has been graphically depicted in Chart V. It will be seen that the lowest average number of fleas was found in October, when two per rat were recorded. From October onwards to April the average number of fleas per rat increased to approximately eight. The greatest number of fleas was

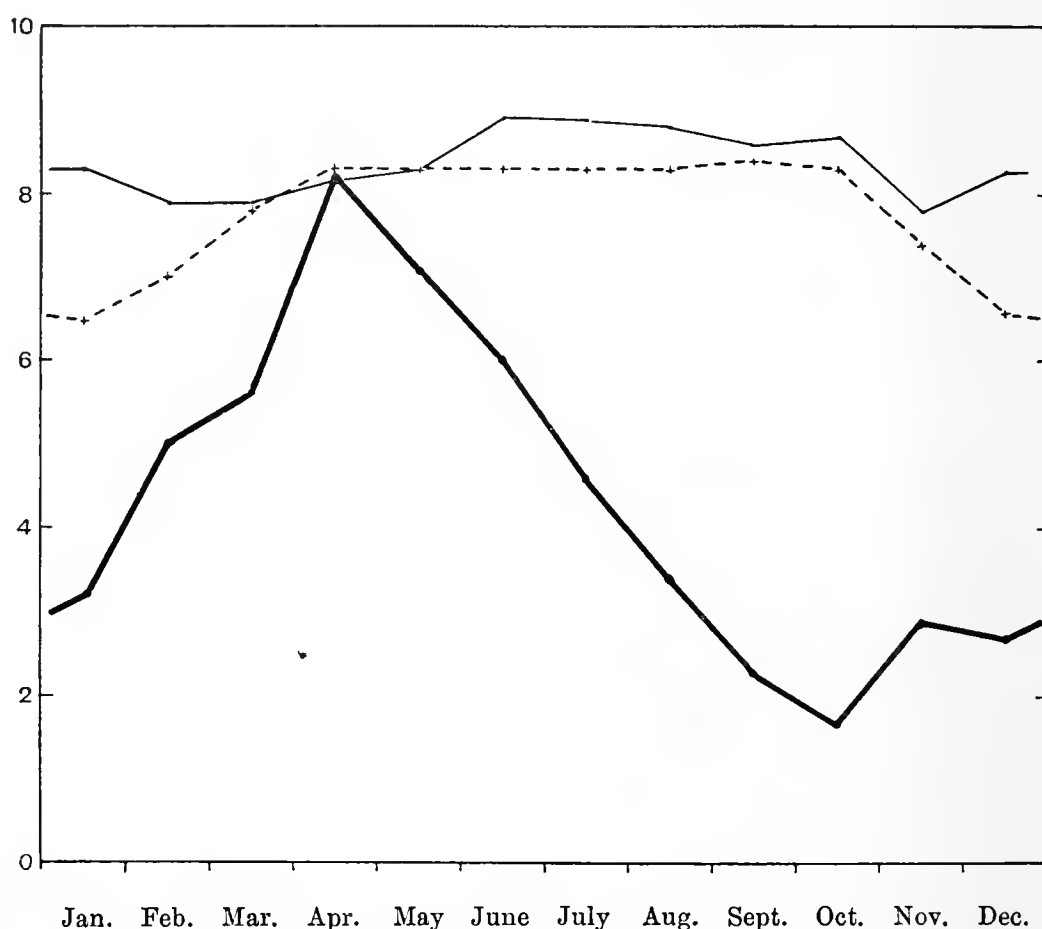


Chart V. Seasonal prevalence of rat fleas in Dacca (•—•).  
Mean monthly temperature (+---+).  
Mean monthly humidity (•—•).

noted in the week ending 10th April when thirteen fleas per rat were recorded. Thereafter a steady decline in the number of fleas occurred till the month of October. It is interesting to note that the curve of the seasonal prevalence of fleas in Dacca (Chart V) corresponds fairly well with the curve of the seasonal prevalence of plague in Calcutta (Chart II).

(II) *Data collected in the district.*

The towns and villages were visited in the following order:—(1) Chittagong, (2) Jatrabari (first trapping), (3) Bhagalpur, (4) Purneah, (5) Raibazaar, (6) Dibrugarh, (7) Jatrabari (second trapping). With the exception of the second trapping of Jatrabari village these places were all visited during the early part of the year 1910 (February to April inclusive), *i.e.* at a time corresponding with the plague season in Bengal. It may be presumed therefore, that, considering the Dacca results, flea prevalence was not below the average at the time each place was examined. Jatrabari village was visited however a second time during the rainy season when the whole surrounding country was under water. This visit was made to find out whether the floods which then covered the country had driven rats into the houses and to see whether we could capture more rats in the houses during the rainy season.

(1) *Chittagong.* (Figs. 19, 20.) This town is an important seaport in the south-eastern portion of the province situated some ten miles up the Karnapauli river. It has a population of 22,140 who live scattered over a considerable area. It is connected with Assam by the main line of the Assam-Bengal railway. Shipping is carried on chiefly with Burmah and Calcutta. Cargo ships come alongside the jetty while passenger ships tie up as a rule in mid stream. In the native city the houses or groups of houses are very scattered. In the middle of the town there are a number of small hills upon which stand the principal offices and the bungalows of the chief European inhabitants. Trapping commenced in the town on the 4th February on the plan described above (p. 162 f.) and was continued till the 12th of the same month with the following results.

Rats caught:

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
65	87	23	45
Average fleas per <i>M. rattus</i>	...	...	3·8
Number of traps set	...	...	620
All rats per 100 traps set	...	..	35·5
<i>M. rattus</i> per 100 traps set	...	..	10·5

(2) *Jatrabari village*. (Figs. 3, 4.) This village is situated on a fairly good road about four miles distant from Dacca town. It was selected as a typical example of a Bengali village. It consists of a long strip of tenements arranged in the shape of the letter **S**, intersected along the middle by the main road; the houses are built on mounds of earth raised above the surrounding country which for a considerable portion of the year is more or less under water. The inhabitants numbered 1325. Trapping commenced on the 18th February and was continued for eight days till the 25th with the following results.

Rats caught :

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
6	46	9	0
Average fleas per <i>M. rattus</i> ...	...	...	2·3
Number of traps set ...	...	...	320
All rats per 100 traps set ...	...	...	19·1
<i>M. rattus</i> per 100 traps set ...	...	...	1·9

The village was trapped a second time from the 18th to the 23rd August. At this time the whole of the surrounding country was under water.

Rats caught :

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
15	23	9	4
Average fleas per <i>M. rattus</i> ...	...	...	0·3
Number of traps set ...	...	...	359
All rats per 100 traps set ...	...	...	14·2
<i>M. rattus</i> per 100 traps set ...	...	...	4·2

(3) *Bhagalpur*. (Figs. 15–18.) With the object of comparing the trapping in Dacca, Chittagong and Jatrabari, which places had never suffered from plague, with a town which had suffered from the disease and which was nearest to Eastern Bengal (Calcutta excepted<sup>1</sup>) a visit was paid to Bhagalpur. This is a large town in Behar. It is situated on the right bank of the river Ganges and has a population of 75,760. It stretches for a length of about six miles and is made up of three portions. It is in railway communication with Calcutta on the one side and the plague-stricken area of Patna and Ballia on the other. Good road and river communication exists in addition between these places. Except for the last two years (1909–1910) epidemics of plague,

<sup>1</sup> Calcutta was not visited because other conditions such as overcrowding, foreign population, etc. come into play. It is an exceptional rather than a representative town for this part of India.

although by no means so severe as in many towns of India, have repeatedly visited the place. The average annual plague mortality for the last ten years per 1000 of population has been 5·5. Trapping commenced on the 27th February and was continued for ten days till the 8th March with the following results.

Rats caught :

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
235	51	22	0
Average fleas per <i>M. rattus</i> ...	...	...	8·1
Number of traps set	...	...	685
All rats per 100 traps set	...	...	45·0
<i>M. rattus</i> per 100 traps set	...	...	34·4

The conditions observed in Bhagalpur were found to differ so remarkably from those found in the towns and villages examined in Eastern Bengal that we decided to examine another town lying between Bhagalpur and the boundaries of the province of Eastern Bengal and Assam and which had not suffered from plague. A town answering this purpose was found in Purneah.

(4) *Purneah*. (Figs. 11, 12, 13.) This town is the capital of the district of the same name. It is situated only 50 miles from Bhagalpur in the old silted up bed of the Kosi river. It had a population of 14,000 in 1901. The inhabitants are scattered over an area of about fifteen square miles and the houses are grouped into three practically distant villages. The part of the town where trapping was carried out consists of one or two main streets which are very wide (as may be seen in fig. 11) with rows of houses, chiefly shops, on each side of the road. Beyond the main streets the houses or groups of houses are sometimes so secluded by trees and widely separated as to be hardly in sight of one another. Trapping commenced here on the 10th March and was continued to the 16th. The results are summarised below.

Rats caught :

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
22	14	54	1
Average fleas per <i>M. rattus</i> ...	...	...	4·9
Number of traps set	...	...	432
All rats per 100 traps set	...	...	21·1
<i>M. rattus</i> per 100 traps set	...	...	5·1

(5) *Raibazaar*. The next place visited was Raibazaar, a large village situated some four miles from Dacca, not far from the river, on

fairly elevated and well-drained land. The village was selected because it was inhabited by a large number of potters who, some generations ago, had come from the town of Rajmhal near Bhagalpur in Behar. The houses in this village differed a good deal from those of an ordinary Bengali village especially in that a few of them had tiled roofs. The population of the village was about 3931. Trapping began on the 20th March and continued to the 3rd of April with the following results.

Rats caught :

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
49	31	16	38
Average fleas per <i>M. rattus</i>	...	...	5.4
Number of traps set	...	...	541
All rats per 100 traps set	...	...	24.8
<i>M. rattus</i> per 100 traps set	...	...	9.1

(6) *Dibrugarh*. (Figs. 21, 22.) The town is one of the largest in Assam and is situated on the left bank of the Brahmaputra river. The population numbered 11,227 in 1901. The inhabitants are Assamese, Bengalis, Beharis, Marwaris and a large number of other races from almost every part of India, who have migrated thither to work on or cater for the extensive tea gardens in its neighbourhood. In the Marwari quarter rows of shops and grain godowns are contiguous with one another along the river bank. Recent floods have destroyed a portion of this quarter and it was here that the small outbreak of plague occurred in 1903. The results of trapping the town are as follows.

Rats caught :

<i>M. rattus</i>	Mice	Musk rats	<i>G. varius</i>
6	9	12	0
Average fleas per <i>M. rattus</i>	...	...	3.5
Number of traps set	...	...	423
All rats per 100 traps set	...	...	6.4
<i>M. rattus</i> per 100 traps set	...	...	1.4

We may conclude from the above experience that except in Bhagalpur the number of rats (*M. rattus*) caught in the towns or villages visited was never greater than eleven for every 100 traps set.

On account of the small number of rats caught (except in Bhagalpur) the flea counts on the rats are not of much value. In the latter place, however, the flea count in the beginning of March, viz. 8.1, was somewhat higher than the count made in Dacca at the same time. It is of

interest to note also that among the fleas found on rats in Bhagalpur about 2% belonged to the species *Ceratophyllus fasciatus*. A single specimen of a new species of flea was captured on a rat in Jatrabari village which has been named by Rothschild *Acropsylla episema* (gen. et sp. nov.)<sup>1</sup>.

	Average <i>M. rattus</i> per 100 traps	Average fleas per rat
Dacca	7	4.2
Chittagong	10	3.8
Jatrabari	{ 2	{ 2.3
	{ 4	{ 0.3
Bhagalpur	34	8.1
Purneah	5	4.9
Raibazaar	9	5.4
Dibrugarh	1	3.5

#### G. DEDUCTIONS TO BE DRAWN FROM THE ABOVE DATA.

(1) *The freedom of Eastern Bengal from plague is due mainly to the scarcity of rats in the houses of the people.*

The number of rats (*M. rattus*) caught in every 100 traps set in Dacca town and in the other towns and villages of Eastern Bengal and Assam visited by the Commission varied from about two in Jatrabari and Dibrugarh to about ten in Dacca and Chittagong. In none of these places, with the exception of Dibrugarh, has bubonic plague ever occurred in epidemic form. Even in Dibrugarh the epidemic, which was of the mildest type, did not last more than a few weeks. In Bhagalpur, which is the nearest plague-infected town to Eastern Bengal (Calcutta excepted), as many as 34 *M. rattus* were caught in every 100 traps set.

In other places in India visited by the Commission *M. rattus* has been caught much more freely. All these places are liable to epidemics of plague.

	Rats per 100 traps	
Belgaum (x. p. 474)	...	13—37; average 23
Poona (x. p. 520)	...	16—43; „ 28
Dhand (vii. p. 912)	...	— ; „ 26
Kasel (vii. p. 913)	...	— ; „ 54
Parel (vii. p. 847)	...	9—65; „ 24
Worli (vii. p. 868)	...	10—70; „ 22

Fleas on the other hand appear to be about as abundant in the province as elsewhere, varying from about two to eight per rat.

<sup>1</sup> N. C. Rothschild, *Novitates Zoologicae*, Vol. xviii. (1911), p. 118.

Comparable figures from plague-infected places previously examined are as follows.

			Fleas per rat
Belgaum (x. p. 461)	...	...	3—19 ; average 10
Bombay (VIII. p. 297)	...	...	2—5 ; „ 4
Dhand (VII. p. 915)	...	...	2—12 ; „ 9
Kasel (VII. p. 915)	...	...	2—13 ; „ 8
Poona (x. p. 525)	...	...	2—9 ; „ 6

In spite of the defects and fallacies of our method of estimating the rat infestation of a place by the number of rats caught in every 100 traps set (fallacies which we have recognised and striven to avoid), we think, in view of the remarkable differences in the figures obtained in this way in Eastern Bengal and Assam and in other parts of India, that we are justified in stating that the main reason for the freedom of Eastern Bengal and Assam from plague depends on the scarcity of rats in the houses of its inhabitants.

(2) *The physical features of the country assist in keeping the province free from plague.*

We have seen that the physical features of the province protect it to some extent from the importation of infection. The nearest plague-infected centres are separated from the province by intervening plague-free areas and the Ganges river. And, although communication by rail with infected centres exists, it is interrupted at places by the great rivers. Roads are few and of an indifferent character while travelling by the rivers and canals although easy is yet slow. Extensive floods during many months of the year still further interrupt free communication between village and village and even between individual homesteads. The villages consist of scattered hamlets built on raised mounds of earth which are spread over an extensive area (see figs. 3 and 4) so that even if plague broke out among the rats in a village infection could only be spread through human agency, the rats in one part of a village being cut off from the rats of another part by long stretches of low-lying ground which is often flooded. The abundant rainfall, ample water supply and fertile soil have prevented famines, so that the people are rich in comparison with those of other parts of India. Sufficient crops are produced for the immediate needs of the inhabitants, so that any trade in grain is in an outward direction while the only imports are of such a kind as are little likely to carry plague infection in the form of infected rats or rat fleas.



H. THE CAUSES OF THE COMPARATIVE SCARCITY OF RATS IN THE  
HOUSES OF EASTERN BENGAL.

We have shown that the freedom of Eastern Bengal and Assam from plague may be attributed chiefly to the small number of rats found in the houses. We must now consider shortly some of the reasons which have brought about this desirable state of affairs.

(1) *The habits of the people.*

We have stated that, owing to the physical features of the country, famines have been entirely absent from the land. The people are therefore richer than those in many other parts of India, and they have accordingly adopted many European habits and customs. Articles of European pattern are frequently to be found in their dress and the use of beds and chairs is common even among the poorer classes. But a striking characteristic of the Bengali is his neatness and tidiness especially compared with the inhabitants of other parts of India. This habit pertains not only to his person but extends to his house and its surroundings. Even within the simple matting huts of the villages one can see pillows, bottles, lamps, utensils and other articles of daily use neatly hung up in a symmetrical manner on the walls. After sweeping and cleaning out the house in the morning nothing unnecessary appears to be left on the floor. How far this habit is due to the experience derived from the floods which occasionally penetrate the houses we are unable to say; it remains, however, a striking feature almost peculiar to the inhabitants of this part of India. But order extends beyond the Bengali home. Around the houses one seldom sees rubbish littered about as is so common in towns and villages in other parts of India. The ground about the house is usually swept, and materials which are of value are generally neatly stacked in piles or bundles. Among many defects in Indian character there is none greater than that which permits him to be satisfied with things that are temporary, tawdry, makeshift or "kutchā." What is good enough for to-day is sufficient for him, he has no consideration for the future. For this reason when an Indian builds a house he generally does so in a haphazard and makeshift manner. When the house is built very little thought is given to its repair, but when this becomes absolutely necessary it is effected by the first materials which come to hand. The Indian seldom

takes any pride in the beauty and neatness of his home. As a result of these slovenly habits the majority of the houses in an Indian village are in a condition representing every stage of dilapidation and ruin.

Fortunately the Bengali suffers less from this defect than the average Indian. He does sometimes take a pride in his home and it is said that one of the greatest assets in the estate of a man of means is a "pucca" family dwelling which can be handed down from father to son. A house is thus built which is expected to last for at least one generation. Where, as in the towns, lime and cement can readily be obtained and wealth is greater a "pucca" house is constructed while, even in the villages, where these materials are scarce, a house is made of corrugated iron imported from Calcutta or is built of neatly spliced bamboo matting with a roof of thatch which is kept in good repair. Nothing can bring home more forcibly the differences between the habits of the Bengali which have been mentioned above and the ordinary Indian villager than a glance at the accompanying photographs. Figures 5 and 6 were taken in a village near Dacca. Figures 13 and 14 were taken in Purneah and Puradah in Bengal. The tidiness, order, symmetry in these villages are in marked contrast to the conditions shown in figures 15 to 18, which were taken in plague-infected Bhagalpur. Here disorder and dilapidation seem to court the presence of the plague.

## (2) *The structure of their houses.*

While the habits of the people may to some extent account for the absence of rats from their homes, yet the construction of the houses leads to the same end. As a matter of fact, whether intuitively or otherwise, the Bengali seems to have adopted a method of house construction which succeeds in practically eliminating rats from his house, be the house a pucca or a kutchra one.

"Pucca" houses, being built of stone or brick or mortar, often with concrete roof, from the very nature of the materials used in their construction afford little harbourage for rats. These animals may however effect an entrance from outside either through the floor or by the door. Many Bengali houses however have been so constructed as to prevent rats gaining an entrance in these ways, being built on raised plinths of special construction. These plinths are erected with a brick or masonry facing, along the upper border of which runs a bead of varying depth which projects for from four to six inches or more over the base. The floor is made of a layer of concrete faced with

hard polished cement, below which is a layer a foot or more deep of dry sand. These devices are said to have been adopted for a number of reasons: (1) to keep the floors dry, a very necessary precaution in a country so subject to floods; (2) to prevent white ants from entering the houses; (3) to prevent the access of rats which cannot make their burrows in the sand or climb over the overhanging plinth. Of course not all the native houses are built in this way but nearly all the new government buildings at Dacca are constructed on this plan.

The "kutchas" houses, too, afford little shelter for rats. For, being constructed of thin bamboo matting or wattle covered with a thin layer of mud, the walls afford no shelter for these rodents. The roofs too, being made of corrugated iron, split bamboo, or thin grass thatch, fail to shelter these animals. Perhaps one of the most noteworthy features of the buildings in this province is the almost complete absence of tiled roofs which are so commonly seen in many other parts of India. Even the houses which are built of mud here differ from those seen in other places. The walls are as thin as possible consistent with stability, being often only ten inches to one foot in thickness. Under these circumstances the entire weight of the corrugated iron or thatch roof has to be taken off the walls by erecting it on four or more bamboo poles. This roof, as will be seen from the photographs 9 and 10, is an entirely separate structure from the mud building; projecting beyond the mud walls, it acts as a shelter protecting them against the sun and rain. The real roof of the building or ceiling of the rooms is made of a thin layer of bamboo battens plastered over with mud. It is said that the chief reason for constructing the houses in this way is to prevent rats burrowing in the walls which are so thin that they fail to find sufficient shelter or security within them. The walls of mud houses in other parts of India are made of such a thickness that rats find ample room to make their burrows within them. Photographs 17 and 18 taken in Bhagalpur demonstrate this fact. Living in this situation the rats not only find a warm and sheltered home but are in close proximity to their food supply which is always abundantly found within and around the untidy Indian dwelling. It will thus be seen that in Eastern Bengal and Assam it is difficult to decide whether "pucca" or "kutchas" houses afford the more shelter for rats; it is evident, at all events, that the structure of the houses here in conjunction with the habits of the people conduce to the comparative scarcity of rats in their dwellings and are the main reasons for the freedom of the province from plague.

Some examples of the various types of buildings found in different parts of Eastern Bengal and Assam are shown in Plates V—XIV. Photograph 7 shows to what extent pucca houses have been built in Dacca town. Photograph 8 shows a group of kutchha buildings in the same place. Photographs 19 and 20 are views taken in Chittagong which illustrate the structural peculiarities of the houses within the town and the suburbs. Photographs 21 and 22 depict the type of houses seen in Dibrugarh in Assam.

#### I. GENERAL CONCLUSIONS.

(1) The province of Eastern Bengal and Assam has suffered very little from bubonic plague; a few epidemics only of pneumonic plague have occurred.

(2) The physical features of the country protect it to some extent from the importation of infection and would tend to limit the opportunities for spreading the disease if it once broke out.

(3) The freedom of the province from plague can chiefly be attributed to the scarcity of rats in the houses as compared with other parts of India.

(4) *M. rattus* is comparatively rare in Bengali houses because of the habits of these people in respect to their greater regard for neatness and tidiness both in and around their dwellings which diminishes the food supply of the rodents.

(5) The structure and design of the Bengali home, whether it be of the solid masonry type on the one hand or of the flimsy matting or grass type on the other, afford little shelter for rats.

(6) The presence of natural enemies of *M. rattus* such as the musk rat may assist in maintaining a low rat infestation of the houses.

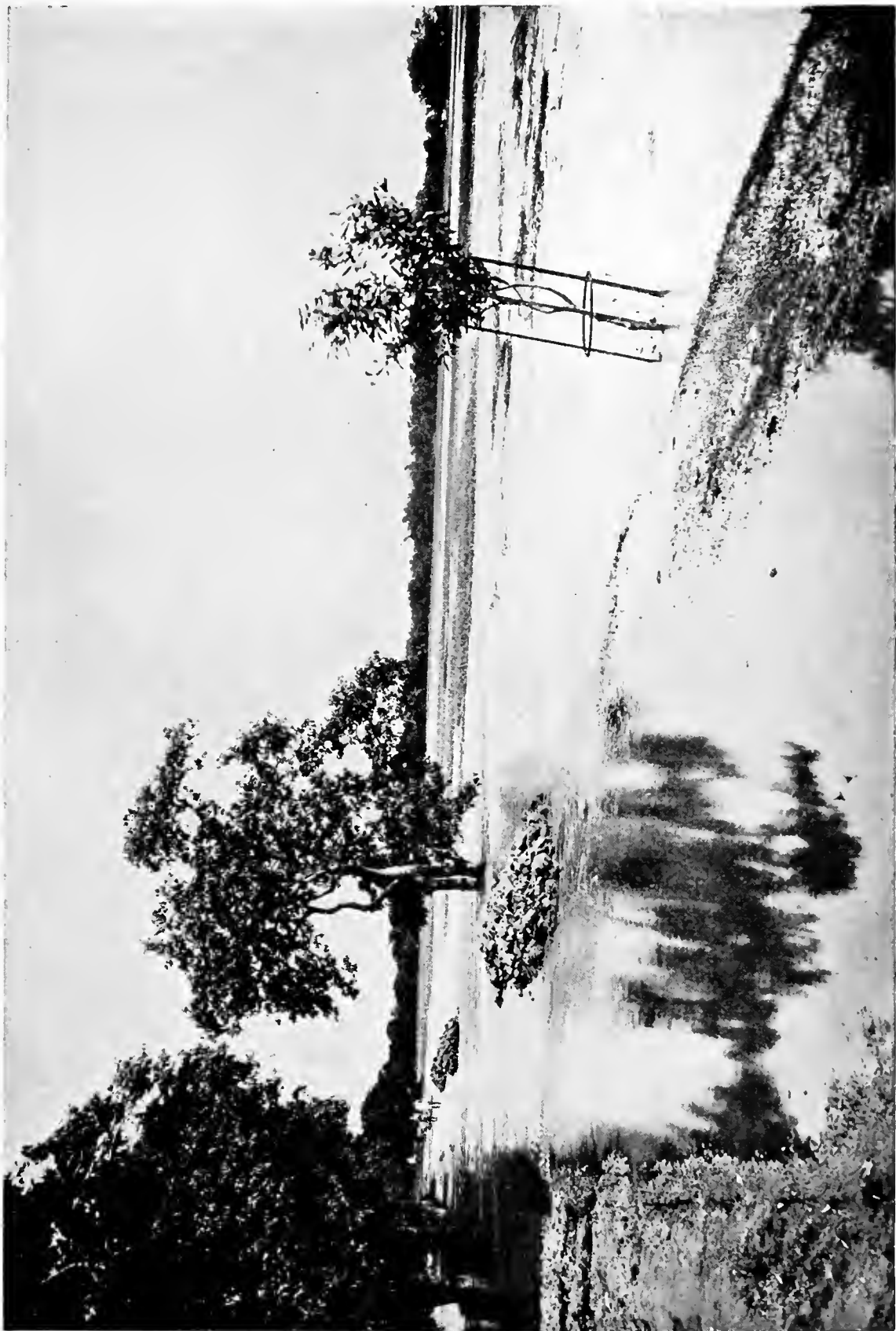


Fig. 1.





Fig. 2.







Fig. 3. Jatrabari village, showing scattered hamlets.



Fig. 4. Jatrabari village: a hamlet.





Fig. 5. Village near Dacca.



Fig. 6. Village near Dacca.



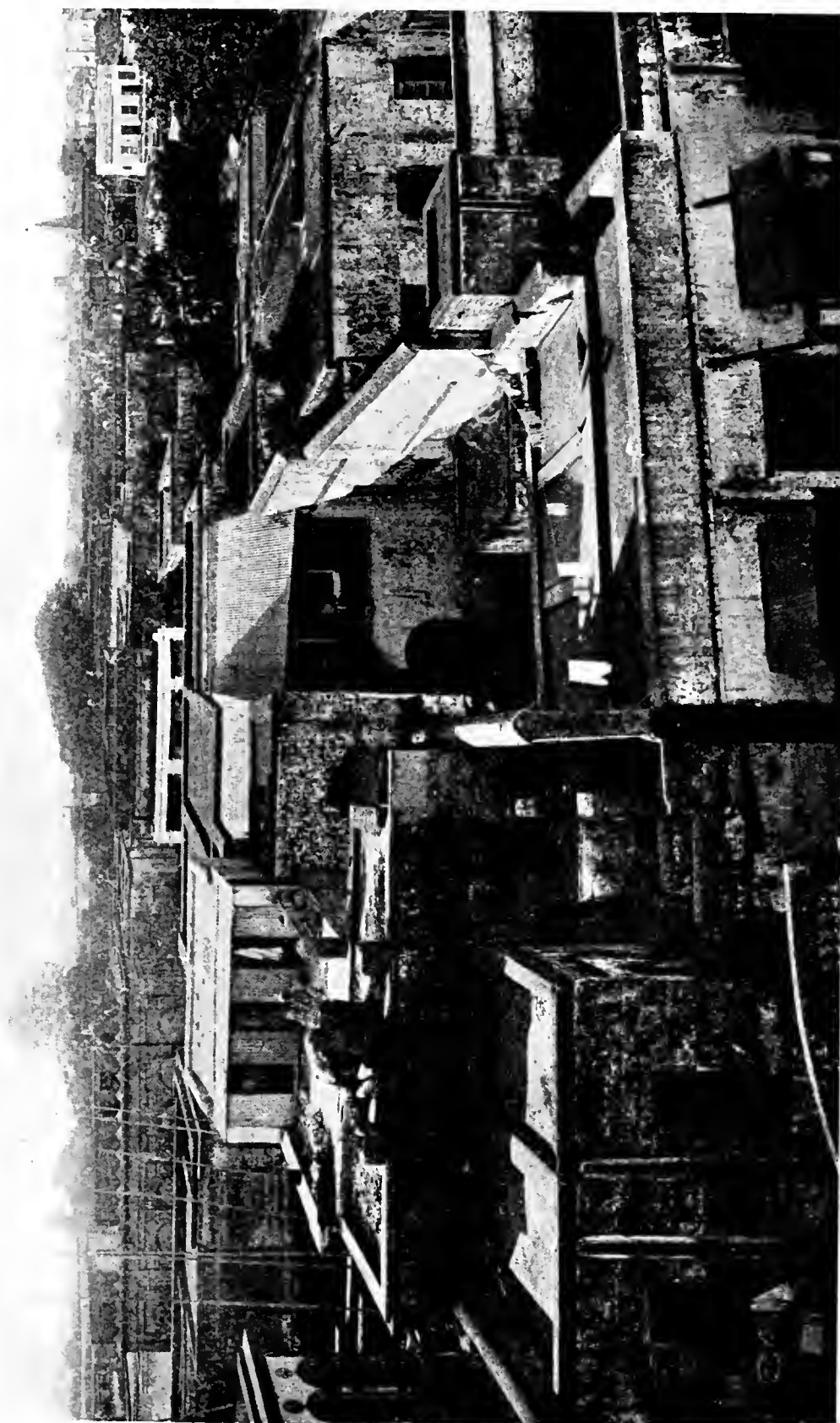


Fig. 7. Dacca: "pucca" houses.







Fig. 8. Dacca: "kutcha" houses.

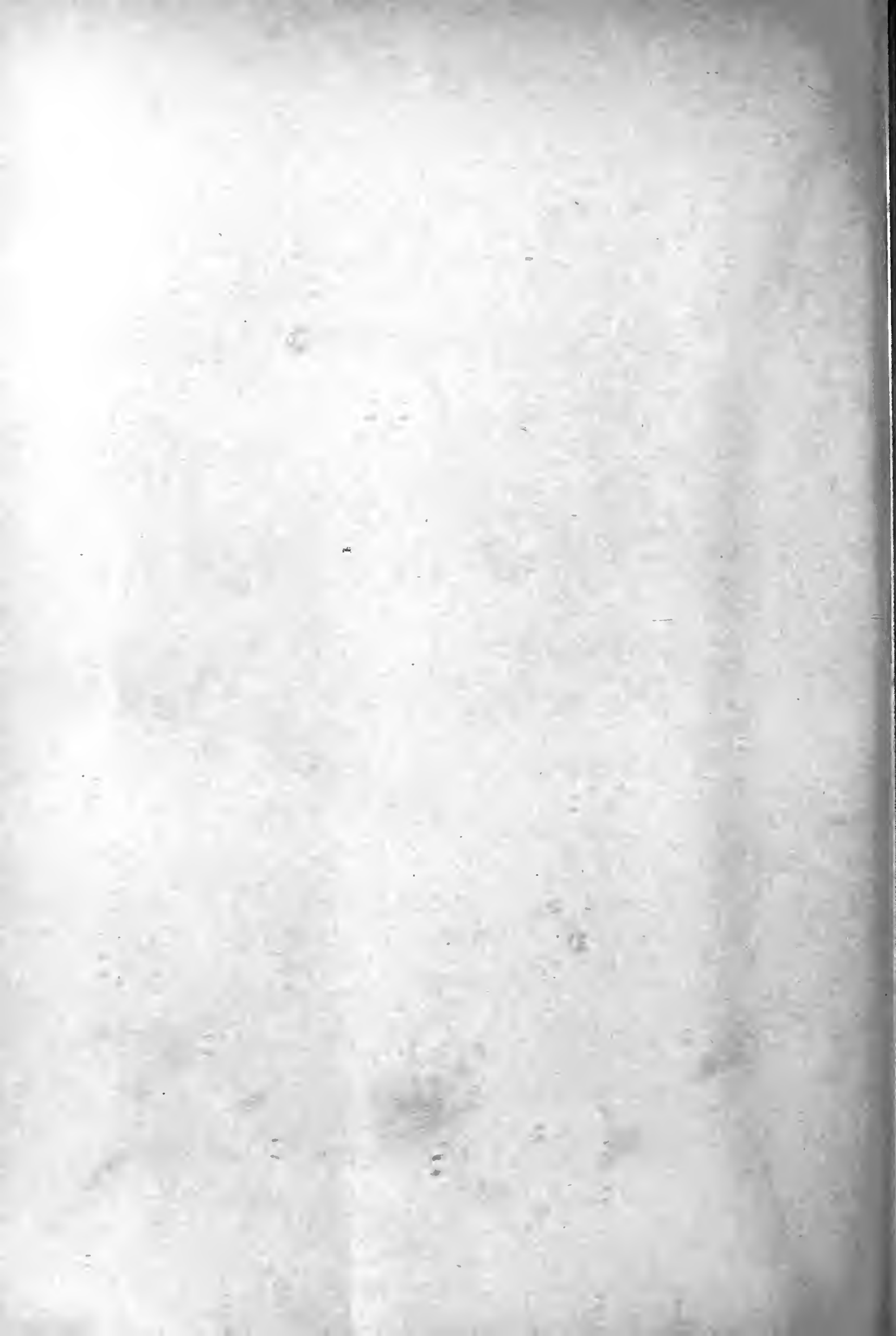






Fig. 9. Showing method of supporting roof: Dacca.



Fig. 10. Showing method of supporting roof: Dacca.





Fig. 11. Purneah: free from plague.



Fig. 12. Purneah: free from plague.





Fig. 13. Purneah: free from plague.



Fig. 14. Puradah, a village in Lower Bengal: free from plague.







Fig. 15. Bhagalpur: plague infected.



Fig. 16. Bhagalpur.







Fig. 17. Bhagalpur: mud walls and rat holes.



Fig. 18. Bhagalpur: mud walls and rat holes.

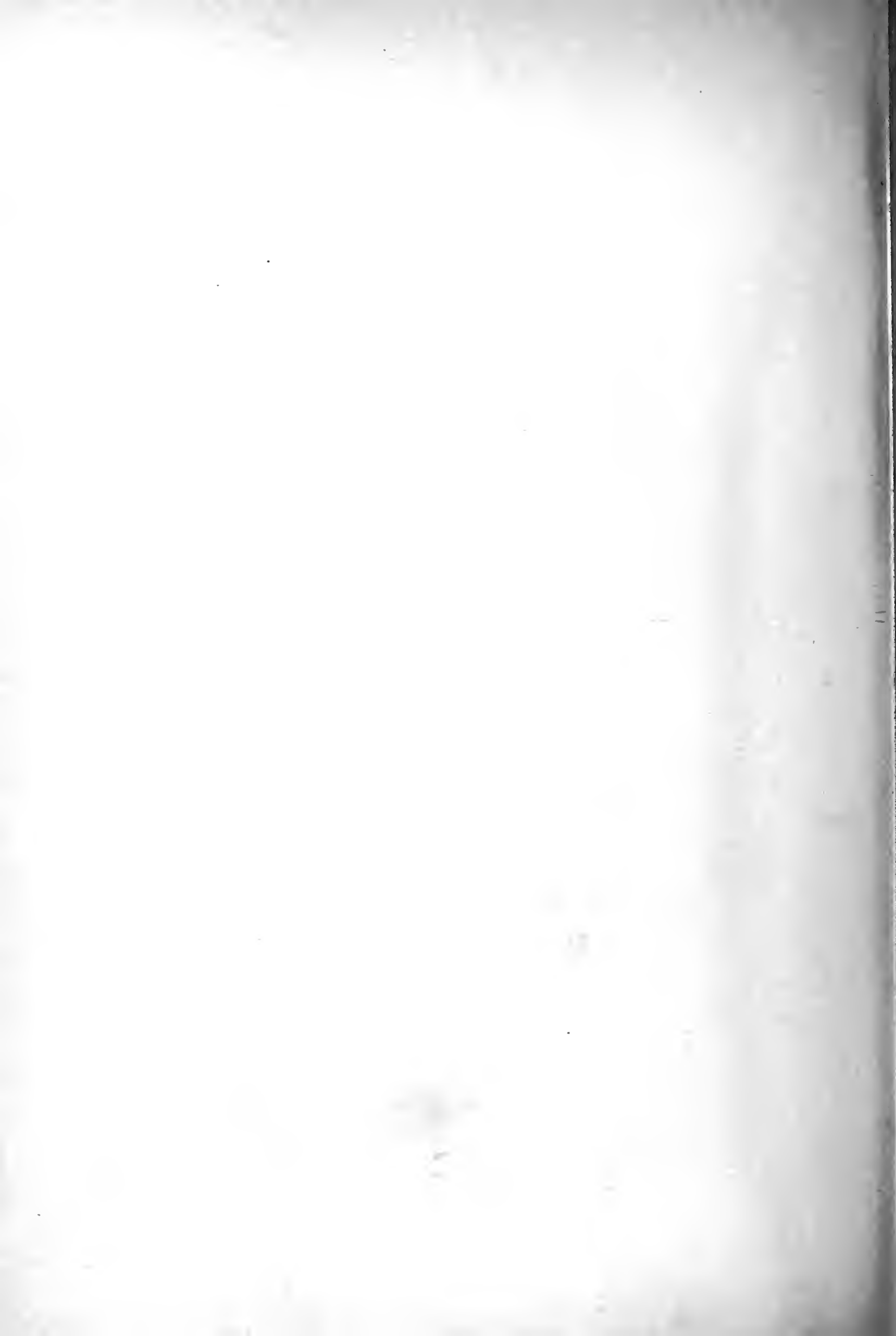




Fig. 19. Chittagong.



Fig. 20. Chittagong.





Fig. 21. Dibrugarh.



Fig. 22. Dibrugarh.



## XLVII. OBSERVATIONS ON THE BREEDING OF *MUS RATTUS* IN CAPTIVITY.

With 1 Chart in Text.

THE present series of experiments on the breeding of *Mus rattus* from wild stock was undertaken in order to obtain reliable data as to the fecundity and breeding habits of this rat. The information was necessary for a proper appreciation of the possibilities of the re-establishment of a rat population after epizootic plague or after destructive measures. The young rats obtained were also required for certain immunity experiments and it was hoped also to obtain some general information as to the rate of growth of rats and other biological facts.

Most of the data available as to the fecundity of rats are based on observations made on *Mus decumanus* (*norvegicus*), but as *Mus rattus* is the one most concerned with the spread of plague in India our breeding experiments were done with this species only.

Attempts to breed from wild *Mus rattus* in cages are generally unsuccessful. These rats will rarely breed at all in cages, and if a litter happens to be born the young ones are usually eaten by their parents within a few days of birth. Knowing this difficulty of breeding in cages, special arrangements were made to breed under conditions in which the natural habitat of *Mus rattus* was more or less satisfactorily reproduced. Six special godowns were built. These were small cabins about six feet square by seven feet high, cement lined and rat and flea proof. In the floor of each godown four small cement lined chambers, with removable lids flush with the floor, were sunk. An artificial burrow consisting of an iron pipe sunk in the floor led into each of these nests. These pipes were about two feet long and of sufficient diameter to admit one rat at a time. A bend in the pipe kept the nest into which it led almost completely dark. Rats let loose in the godowns could run about the floors or climb on the metal work of the double doors or retire down the pipes to their nests, and, in general, have very much the same freedom of movement as under natural wild conditions.

As will be seen, breeding was successfully accomplished in these godowns and a considerable number of litters were reared.

On the 20th July, 1909, the following pairs of rats were put into the godowns :

Godown No. 1	...	...	One pair.
" " 2	...	...	Two pairs.
" " 3	...	...	Four pairs.
" " 4	...	...	Eight pairs.
" " 5	...	...	Sixteen pairs.
" " 6	...	...	One pair.

The result of this arrangement was as follows :

*Godown No. 5 : 16 pairs.* Up to 2nd Sept. 1909 eight males and eight females died, probably from fighting, many of them being eaten. The remaining eight pairs were then killed and examined but none of the females were found pregnant. Four fresh males and twelve females were then put in and left until 25th Sept. when they were removed to make room for a successfully breeding pair from godown No. 6. None of the twelve females were pregnant.

*Godown No. 4 : 8 pairs.* Four males died up to 20th Sept. The number was then reduced to one pair. None of the females removed were pregnant.

*Godown No. 3 : 4 pairs.* A litter of six was born on 13th Sept. but was eaten on 17th Sept. The number was reduced to one pair on 20th Sept. None of the females were pregnant.

*Godown No. 2 : 2 pairs.* One male died on 13th Sept. and one female on 17th Sept. One pair was thus left which commenced breeding, a litter being born on 15th Oct.

*Godown No. 6 : 1 pair.* A litter of five was born on 2nd Sept. On 25th Sept. the parents were removed to godown No. 5.

*Godown No. 1 : 1 pair.* The female killed three successive males on 8th Aug., 18th Aug., and 11th Nov. She was removed on 11th Nov. and kept till 6th Dec. and then killed and found not pregnant.

It will be seen that the first litter to be successfully reared was in godown No. 6 with only one pair of rats. The second litter successfully reared was in godown No. 2 which originally contained two pairs but in which breeding did not take place until after the death of one pair. The only litter born in the godowns containing more than one pair was in godown No. 3 and the young ones were eaten within four days of birth. The failure to breed of the pair in godown No. 1 was probably due to the female being old and unsuitable.

It thus appears that the most satisfactory arrangement to obtain successful breeding is with only one pair in each godown. From 20th Sept. 1909 onwards this arrangement was adopted and numerous litters were reared successfully.



BREEDING HISTORY OF SUCCESSFUL PAIRS FROM  
20TH SEPT. 1909 ONWARDS.

*Pair in Godown No. 2.*

The following litters were born :

15th Oct. 1909	3 born	Male removed.
17th Nov. „		Male returned to godown.
11th Dec. „	6 born	Male not removed.
13th Dec. „		Two young ones eaten. Male removed.
10th Jan. 1910		Male returned to godown.
13th Feb. „	6 born	Some showed tooth marks. Male removed.
14th Feb. „		Female has eaten one.
21st Feb. „		One more eaten by female, head only left.
1st Mar. „		Male returned to godown.
4th Mar. „	6 born	Male not removed.
5th May „	5 born	Male removed.
2nd June „		Male returned to godown.
10th Aug. „	8 born	All eaten on the same day.

*Pair in Godown No. 3.*

The following litters were born :

9th Nov. 1909	6 born	Male removed.
27th Nov. „		Male returned to godown.
18th Jan. 1910	8 born	Male not removed.
31st Mar. „	7 born	Male removed
20th Apr. „		Male returned to godown.
25th May „	5 born	Male not removed.
10th Aug. „	7 born	„ „
8th Oct. „	6 born	„ „
30th Dec. „	4 born	„ „

*Pair in Godown No. 4.*

Put in on 6th Dec. 1909. The following litters were born :

7th Feb. 1910	6 born	Male removed.
26th Feb. „		Male returned to godown.
20th April „	5 born	Male removed.
25th April „		Two eaten by female.
26th April „		Three eaten by female. Male returned to godown.
23rd June „	6 born	Male removed.
25th June „		Whole litters eaten by female.

Pair killed off on 29th Sept. 1910 and one fresh male and two females put in.

10th Feb. 1911	7 born	Male not removed.
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*Pair in Godown No. 5.*

The following litters were born :

2nd Sept. 1909	5 born	This litter was born in godown No. 6 and was transferred to this godown on 25th Sept. 1909. Male not removed.
6th Nov. ,,	1 found	This rat was apparently several days old and was possibly one of a litter several of which may have been eaten. Male not removed.
20th Nov. ,,	5 born	Male not removed.
3rd Jan. 1910	5 born	,, ,,
21st Feb. ,,	3 born	,, ,,
2nd May ,,	5 born	,, ,,
30th June ,,	5 born	,, ,,

Pair killed on 29th Sept. 1910.

*Pairs in Godowns Nos. 1 and 6.*

No litters were obtained.

## SECOND SERIES OF GODOWNS.

A second series of breeding godown was started on 19th Oct. 1910, one male being placed in each godown with one or more females. A few litters have been obtained.

*Godown No. 7.*

One male and three females.

16th Dec. 1910	litter born	Number unknown as all were eaten up with the exception of parts of heads and tails.
8th Mar. 1911	6 born	Two females and the male removed.

*Godown No. 8.*

One male and one female.

17th Jan. 1911	4 born	Male removed.
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*Godown No. 9.*

One male and two females.

3rd Mar. 1910	5 born.
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*Godown No. 10.*

One male and two females.

No breeding.

*Godown No. 11.*

One male and three females.

7th Feb. 1911	5 born	Mother and young removed.
12th Feb. „		All eaten by mother.
12th Feb. „	7 born	From second female.

*Godown No. 12.*

One male and three females.

No breeding.

*Breeding of a second generation.*

A male and female from separate litters born in the godowns were placed in a large specially constructed cage which contained a box lined with straw in imitation of the breeding chambers of the godowns.

The male of this pair was from the litter born in godown No. 2 on 15th Oct. 1909 and the female from the litter born in godown No. 5 on 2nd Sept. 1909.

The following litters were born :

19th Feb. 1910	7 born	Male removed.
2nd Mar. „		Six eaten by female, remaining one removed and male returned to cage.
30th May „	6 born	Male not removed.
17th Oct. „	6 born	„ „
2nd Jan. 1911	6 born	Male removed.
11th Jan. „		Four eaten by female, remaining two dead. Male returned.
13th Feb. „	8 born	

The results obtained in the most successful of these godowns supply us with figures from which we can get a fair idea of the fecundity of *Mus rattus*. Of course the temporary removal of the males in some of the godowns, after the birth of a litter, may have prevented the females again becoming pregnant as soon as might otherwise have happened, and the frequency of litters was perhaps diminished in this way. The artificial conditions may also have diminished the natural rapidity of breeding.

A short summary of the litters obtained from pairs giving three or more litters will provide the figures on which to base a calculation of fecundity.

*Rat breeding**Pair in Godown No. 2.*

Date	Number born
15th Oct. 1909	3
11th Dec. „	6
13th Feb. 1910	6
4th Apr. „	6
5th May „	5
10th Aug. „	8

Total 34 born in 9 months and 26 days.

*Pair in Godown No. 3.*

9th Nov. 1909	6
10th Jan. 1910	8
31st Mar. „	7
23rd May „	5
10th Aug. „	7
8th Oct. „	6
30th Dec. „	4

Total 43 born in 13 months and 20 days.

*Pair in Godown No. 4.*

Date	Number born
7th Feb. 1910	6
20th Apr. „	5
23rd June „	6

Total 17 born in 4 months and 13 days.

*Pair in Godown No. 5.*

20th July 1909	5
6th Nov. „	1
20th Nov. „	5
3rd Jan. 1910	5
21st Feb. „	3
2nd May „	5
30th June „	5

Total 29 born in 11 months and 10 days.

*Pair in Breeding Cage No. 1.*

19th Feb. 1910	7
30th May „	6
17th Oct. „	6
2nd Jan. 1911	6
13th Feb. „	8

Total 33 born in 11 months and 22 days.

*Rate of increase of rats.*

Working from these figures showing the number of young born in a given period, we can calculate roughly the number of young which an actively breeding pair will produce in a year. Allowing three weeks for the first incubation, the following approximate figures are obtained for four successful pairs :

Pair in Godown No. 2	39 in 12 months.
„ „ No. 3	36 „
„ „ No. 4	44 „
„ „ No. 5	29 „
„ Cage No. 1	32 „

This gives an average of 36 in twelve months or a litter of six (the commonest number) every two months.

The following figures show the number of rats in our litters :

Litters of one	...	...	doubtful
„ two	...	...	nil
„ three	...	...	2
„ four	...	...	2
„ five	...	...	10
„ six	...	...	12
„ seven	...	...	5
„ eight	...	...	3
Average litter 5.7.			Commonest litter 6.

These figures may further be applied to the calculation of the total progeny of a pair in a given period allowing for the coming to maturity and the commencement of breeding of successive generations of young rats. We assume that each litter consists of six, and we take it for granted that a litter can be produced when the rats reach the age of five months. The age of five months as the starting point for breeding is based on the pair, details of whose breeding of a second generation have already been given. The male of this pair was four months old at the time of the birth of the first litter, and the female  $5\frac{1}{2}$  months. The rats however were, to external appearances, sexually mature by the end of the third month, and under natural conditions would probably breed at an earlier period than five months. In the table which Boelter gives for *decumanus* the first litter is taken as being born when the parents are  $3\frac{1}{2}$  months old.

Our table also assumes that at least as many females are born as males. Our figures show more females than males to have been born, and also that polygamy occurs. It will thus be seen that the figures taken err, if anything, on the side of under estimation. The calculations show that a single pair may produce 198 young in 12 months and 858 in 16 months. This does not allow for destruction of young by their parents or by natural means.

*Intervals between the births of litters.*

When the male had continued access to the female, the following intervals occurred between two litters:

29 days, 31 days, 44 days, 47 days, 49 days, 70 days, 72 days, 80 days, 82 days.

Average interval—56 days.

When the males were removed for varying periods the following intervals elapsed between re-introduction of the male and a birth:

24 days, 33 days, 42 days, 50 days, 53 days, 58 days, 78 days.

Average interval—48 days.

Out of 55 born in the godowns

24 were males (43·6 %)

31 were females (56·4 %)

For comparison we give the percentage of the sexes of wild rats caught in Bombay (calculated on over 30,000):

Males 51 %

Females 49 %

*Polygamy.*

The results in godown No. 11 show definitely the occurrence of polygamy. In this godown there was one male with three females. Two of the females gave birth to a litter within five days of each other, the one litter consisting of five and the other of seven.

*Eating of young rats by the parents.*

Out of 35 litters, ten were eaten by the parent rats. Of these ten litters, six were eaten by the female alone and the remaining four litters were eaten by either the male or female. The extent to which this destruction of young goes on under wild conditions is unknown. In our godowns destruction may have been unnaturally large owing to the handling of the young rats for the purpose of weighing and measuring and to the absence of flesh diet. Throughout the whole period of the experiments the only food in the godowns was parched rice or radish. No flesh was given at any time. The rats remained healthy and fat on this diet. There may, however, have been a craving for flesh which led to the eating of the young, and it has frequently been observed that rats in cages will eagerly devour raw flesh or other rats when they will entirely refuse to touch vegetable food. Handling could not have been a factor in many cases for some litters were eaten which had never been touched. It is possible that the same destruction goes on in nature even when some flesh food is available, but we can have no accurate guide as to its extent.

## RATE OF GROWTH OF YOUNG RATS.

Records of weights, head and body lengths and tail lengths were kept for several litters for varying periods up to four months after birth. The tails were sometimes broken off in handling or bitten off by the rats themselves when fighting, and for this and other reasons the measurements could not be continued as long as might have been desired. The main facts, however, as to rate of growth are shown in the following tables which give the averages for each litter.

TABLE I.

*Average weights and measurements of young rats.*

Litter of six born 9th Nov. 1909.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·6	—	—
6	10·8	59	41
13	20·0	80	83
16	22·8	—	—
20	23·0	96	115
27	27·5	104	132
34	38·3	110	149
41	40·5	117	151
48	48·5	134	160
55	57·0	137	164
62	65·0	140	170
69	70·3	143	173
76	76·0	146	177
83	77·5	—	—
90	83·3	—	—
97	84·0	—	—
104	86·6	—	—
111	92·1	—	—
125	98·6	166	188
139	100·0	167	188

TABLE II.

*Average weights and measurements of young rats.*

Litter of five born 20th Nov. 1909.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·2	—	—
1	5·0	49	22
2	5·8	—	—
3	6·6	—	—
5	9·0	—	—
9	14·0	70	56
12	17·4	—	—
13	—	83	76
16	21·6	87	95
23	25·8	98	118
30	31·0	108	133
37	40·0	122	143
44	50·0	126	153
51	58·0	129	165
58	66·6	139	169
65	71·8	143	178
72	82·0	144	186
79	89·0	149	190
86	96·6	155	192
93	98·6	—	—

TABLE III.

*Average weights and measurements of young rats.*

Litter of six born 11th Dec. 1909.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4.1	41	20
2	6.0	48	22
4	7.7	57	30
6	10.7	60	35
9	16.0	69	48
16	27.7	94	92
23	40.0	118	123
30	54.0	126	155
37	59.2	130	165
44	63.5	140	170
51	74.0	143	175
58	82.2	146	180
81	110.6	158	199

TABLE IV.

*Average weights and measurements of young rats.*

Litter of five born 3rd Jan. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4.2	44	18
1	5.0	—	—
2	5.8	49	24
4	8.0	62	33
5	9.2	—	—
7	11.4	67	44
9	13.8	72	53
11	16.4	77	64
14	19.4	86	85
17	22.0	91	103
21	27.0	101	122
23	28.0	103	127
28	28.0	104	133
35	36.0	108	143
42	42.0	117	150
49	49.7	128	157
56	60.5	133	166
63	65.8	136	174
70	74.0	145	183
77	82.2	147	185
105	115.0	165	192



TABLE V.

*Average weights and measurements of young rats.*

Litter of six born 18th Jan. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4.1	45	22
1	4.5	—	—
2	5.2	52	26
3	6.1	—	—
6	9.3	63	40
8	11.5	68	47
11	14.6	77	65
13	16.6	87	87
20	24.1	94	113
27	29.0	104	139
34	33.0	115	147
41	38.7	118	149
48	47.2	125	158
55	49.8	131	165
62	57.0	134	168
69	63.0	142	175
76	64.0	143	178
83	67.5	144	178
90	68.2	147	180

TABLE VI.

*Average weights and measurements of young rats.*

Litter born on 7th Feb. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4.7	44	20
2	5.5	51	23
4	7.0	57	29
7	9.1	65	40
9	10.6	68	47
11	12.0	74	58
14	14.1	80	68
19	16.0	79	99
28	22.2	98	103
35	27.0	108	117
42	32.6	113	128
49	39.0	123	134
56	51.6	128	147
63	58.1	138	160
77	76.0	141	162

TABLE VII.

*Average weights and measurements of young rats.*

Litter of three born 21st Feb. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4.0	48	22
2	6.0	53	27
5	10.0	62	45
7	13.3	71	53
11	20.0	82	78
14	24.0	90	96
Removed from mother and put in cage No. 17.			
21	22.0	101	109
28	27.3	105	137
35	32.0	114	145
42	40.0	117	155
49	43.3	125	164
56	51.0	128	165
66	55.3	134	168

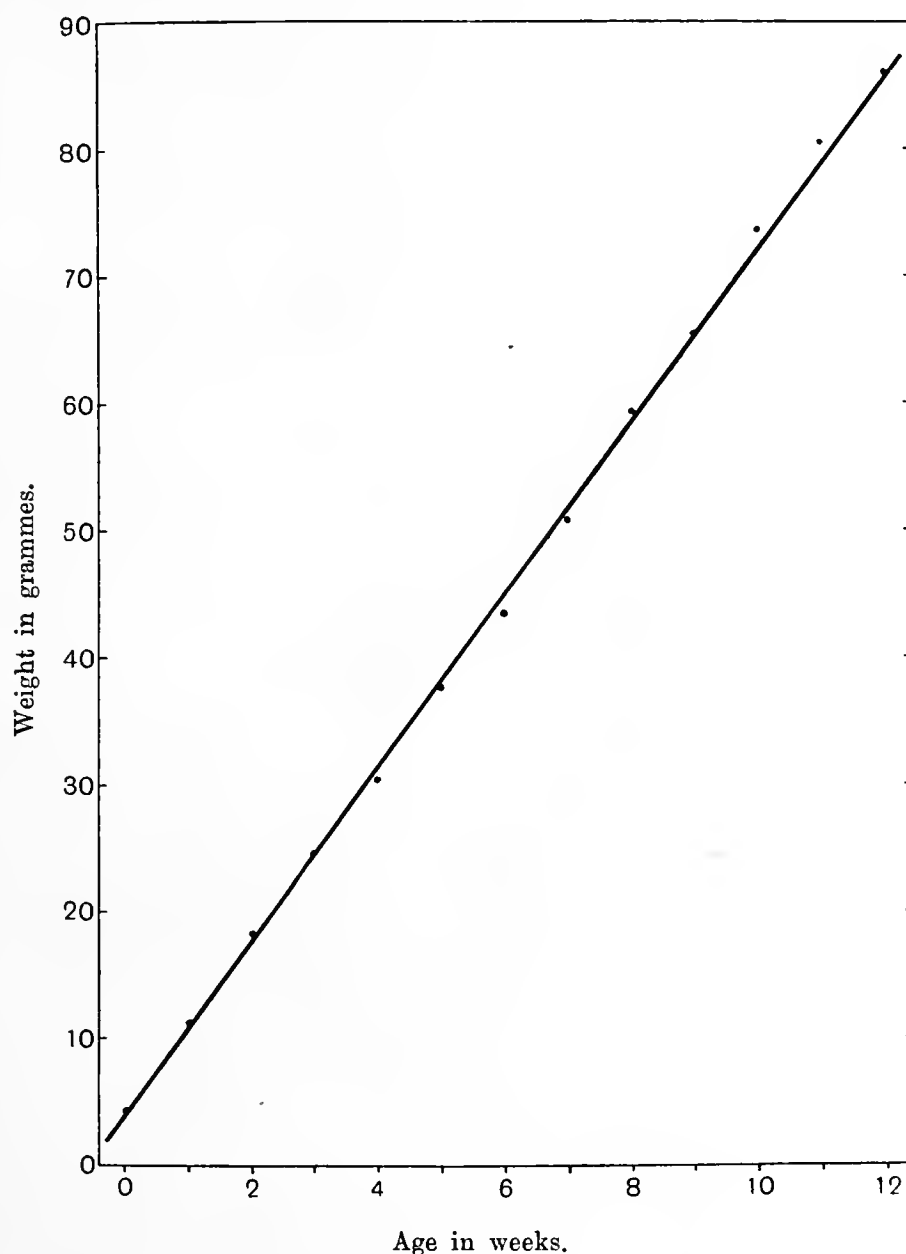
TABLE VIII.

*Table of average weights calculated from seven litters.*

Age	Weight
At birth	4.3 grammes
1 week	11.3 „
2 „	18.3 „
3 „	24.7 „
4 „	30.4 „
5 „	37.7 „
6 „	43.4 „
7 „	50.9 „
8 „	59.4 „
9 „	65.5 „
10 „	73.6 „
11 „	80.4 „
12 „	86.0 „

*Weight.* It will be seen that the weight at birth varied from 4 grammes to 4.7 grammes, the average of seven litters being 4.3 grammes. The rate of increase of weight was approximately 1 gramme per diem up to the end of three months, beyond which period our figures are scanty. This regular increase is shown in all our tables, Table II and Table IV conforming very closely to the average growth of 1 gramme

per diem. Table III considerably exceeds this while the others are slightly under it. The weights in Tables I to VII have been used to make a composite table of weights, Table VIII, in which the average weekly growth has been calculated.



The weight of a rat in grammes, minus 4 grammes for weight at birth, would according to our observations give an approximate idea of the age of the animal in days, up to a weight of about 100 grammes. This calculation would not hold for many of our rats. For example, we have one rat weighing 82.2 grammes at 58 days, while another weighs 83

grammes at 90 days. Again, one rat weighed 100 grammes at 139 days and another 138 grammes at 123 days. On an average the calculation would however probably be a fair approximation.

Observations on many thousands of wild rats show that the majority fall into the weight groups between 100 grammes and 110 grammes. The ordinary adult rat picked at random for experimental purposes is usually of this weight and we may take it that a *rattus* of this weight may be called "grown up." *Mus rattus* therefore probably becomes "grown up" sometime in the fourth month.

Some of the tables show a diminution of the rate of growth towards the end of the third week. This is probably due to the cessation of suckling and the change to a vegetable diet. Table VII shows the result of removing the young from their mother too soon. The young rats were removed at the 14th day, and instead of gaining weight during the following week they lost an average of 2 grammes each. They continued to grow in length during this week but became very thin. They eventually became accustomed to the new diet and picked up weight. Suckling probably goes on well up to the end of the third week.

The young rats usually have their eyes open between the 11th and 15th days, the 14th being the commonest day.

Tame white rats<sup>1</sup> grow a good deal faster than these *rattus*, possibly because they may ultimately become larger. According to Donaldson's figures<sup>2</sup>, starting with a weight of about 5 grammes at birth they weigh 15 after a fortnight and 30 when a month old; at 7, 10 and 12 weeks the average weights are 57, 103 and 135 grammes respectively.

*Length.* At birth the head and body length varies from 41 to 48 millimetres and the tail length from 18 to 22 millimetres. The tail is thus only half the length of the head and body at birth. The growth of the tail is at first very rapid and it becomes equal in length to the head and body within 12 to 16 days. At the end of the first month the tail has attained its proper length in proportion to the head and body of 25 % more, and afterwards maintains this proportion.

<sup>1</sup> According to Hatai (*Biological Bulletin*, Vol. xii. (1907), p. 266) these are *Mus norvegicus* (*decumanus*).

<sup>2</sup> *Boas Memorial Volume*, 1906, p. 5. For *Mus decumanus* see H. H. Donaldson and S. Hatai, *Journ. Comp. Neurology*, Vol. xxi. (1911), p. 417.

## XLVIII. PLAGUE IN MADRAS CITY.

6 MADRAS City, in common with most places in the province, has suffered relatively slightly from plague. With the exception of a single epidemic in 1905-6, plague infection has never gained a foothold in the town, despite the fact that opportunities for importation appear to have been abundant since 1896, when plague first began to prevail in Bombay. The present enquiry was undertaken in order to throw light on this apparent immunity. Reducing the matter to its simplest terms, Madras City may be presumed to have escaped infection either because plague cannot reach the place or because the circumstances obtaining there are unfavourable to the propagation of imported infection. The practical part of our investigations has been for the most part directed towards an examination of this latter question especially as regards the circumstances of the rat and flea population of the town.

1. *Introduction.*

Madras is the third largest city in India and has a population of a little over half a million people (509,346). It is built in straggling fashion on a strip of land nine miles long and of an average breadth of about three miles. It is low-lying and flat. Though possessing little more than half the population of Bombay, the area of Madras is five square miles larger than the area of Bombay. It is quite unlike the other presidency towns of India and can best be described as a collection of small towns and villages separated one from the other by considerable tracts of open country. In some of the native quarters of Madras there is much crowding—Georgetown, Triplicane, Chintadripetta, for example, are very densely populated. In the middle of Georgetown the number of persons living in each inhabited house is thirteen. Single storied houses are the rule; double storied dwellings are common in some portions of the town.

It is difficult in a few words to convey any idea of the house construction in the native quarters of a city such as Madras when one sees all types of dwelling from the simple mud hut with mud floor and tiled or thatched roof to the solidly built two storied dwelling

of the well-to-do inhabitants. Let it suffice to say that, from the point of view of rats and plague, the best houses, built as they are on high stone plinths, would be difficult to improve on, whereas the poorer houses afford unlimited shelter for rats. There certainly appears to be nothing in the construction of large portions of the town that can explain its immunity from plague, and, as we shall presently see, rats are present in abundance everywhere.

*Risk of importation of plague by sea.* Madras is a seaport of importance and ranks fifth among the Indian ports in the value of its trade. Imports exceed exports in value. Most of the imports come from the United Kingdom (70 per cent.), but a not inconsiderable amount of rice comes from Rangoon, with which place Madras is in close communication by sea. There is a large passenger trade between these two ports, and Rangoon, on both these counts, is a constant menace to Madras owing to the fact that plague is usually prevalent in the former port. Madras is an artificial harbour and until recently afforded no facilities for boats of large burthen coming alongside the wharfs. The ships are anchored some way from the quays and the cargoes are discharged into small boats in which they are taken ashore. This method of disembarking cargo diminishes, though it does not obliterate, the risk of importing plague-infected rats with grain. The rice is imported packed in gunny bags, and stored for varying periods of time in large godowns close to the docks, until disposed of by the importers. Third class passengers by steamer from Rangoon have their luggage opened and disinfected by heat. They are not compelled to submit the clothes they are wearing to disinfection. All passengers from Rangoon are passported as described below.

*Risk of importation of plague by rail.* Two railway systems, the 'Madras and Southern Mahratta' and the 'South Indian,' link up Madras with the rest of the Indian Peninsula. A northern line links up Madras with Calcutta, a southern line with Tuticorin, a north-west line with Bombay and a south-west with the west coast of the Presidency and Mysore. It is the last mentioned line that is the chief danger to Madras, for it is by means of it that the city is brought into closest communication with districts that are always plague-infected.

Most of the grain and pulse that is imported by land comes from districts that are free from plague, viz. Ganjam, Vizagapatam, Godavari, Nellore, Chingleput and North Arcot. Most of the risk, therefore, that Madras City runs of becoming plague-infected is by railway passenger

traffic. To meet this risk all passengers arriving in Madras from plague-infected districts are passported. Every passenger is bound to take out a passport and has to report himself every day for a period of ten days to one of the passport examining offices in the city. No disinfection of any of the passengers' luggage is carried out, and merchandise travels unrestricted. Associated with the passport system is a very complete and vigorous sanitary organisation.

As an illustration of the exposure of Madras City to plague infection, we may note its relations with Bangalore in the native state of Mysore. Bangalore is distant from Madras about 190 miles and the passenger traffic between the two places is extensive. In the years 1901—1909, in a population of about 70,000, there were 15,400 deaths from plague, and in no month during that period did the number of plague deaths in Bangalore fall below 10.

## *2. Plague in Madras City.*

Madras City has suffered from one mild outbreak of plague. Happily this occurred on the outskirts of the town, where it was possible to deal with it energetically. The following description of the outbreak is from a report written at the time by Major Ross and filed in the office of the Health Department of the Madras Municipality:—

Indigenous plague was first discovered in Madras on 20th January, 1905. On this date, the attention of a Sanitary Inspector having been drawn to the more than usual number of deaths in a fishing village (No. 1) on the northern outskirts of the town, a careful house-to-house enquiry was made, with the result that three females were found suffering from sloughing "ulcers" in the groin. These cases were diagnosed as plague, and were of course at once isolated.

The village consisted of 118 huts, with a population of 613, and was situated on the sea coast, with immediately on its rear the Mauritius-Fiji Emigration Depot enclosed on that and two other sides by high brick walls. A careful search was made in the infected village for dead or live rats, but none could be found in the huts. On the road between the village and the depot wall a dead rat was found on the 22nd January, and on passing the same spot about two minutes afterwards another rat some time dead was found, having evidently been thrown there by someone, most likely from inside the depot. Enquiries were at once made of the Depot Superintendent, and he admitted that rats had been dying within the depot, but he did not know of what cause or how they were disposed of. The rats on bacteriological examination at Guindy were pronounced plague infected and the depot was at once evacuated, though strangely enough no human plague cases were ever reported from amongst the emigrants with whom the depot was at the time crowded. Whether the emigration depot with its hundreds of coolies from plague-infected areas or the fishing village with its harbour-working

population was the first to become infected was impossible to prove, as the first human case had occurred a fortnight before discovery and rat infection in a place where the sweeper was allowed to dispose of the dead rats by throwing them over the wall into the village may have been going on for any length of time.

The fishermen were at once moved into a new village provided by the Municipality a few hundred yards distant and the old village was burned down. A line of coolies was placed around the village during the burning to prevent the escape of rats, but as the work was done after dark it is just possible that some may have escaped. Just before evacuation on 24th January, 1905, another case of plague was discovered in the village, and none have occurred since, though they have long ago been allowed to reoccupy the old site. No further cases occurred in the neighbourhood until 18th February, 1905, when a very suspicious death occurred in a small village (No. 2) just south of and a few yards distant from No. 1, and on 23rd February two cases were discovered in another village (No. 3) a few yards west of No. 2. On 24th February another case occurred in No. 2 village, and on 24th and 25th three cases occurred in village No. 3. These villages were similarly dealt with to No. 1, and during the burning down coolies were posted to prevent the escape of rats; one, however, did make good his escape.

Immediately it was discovered that the rats had become infected, a rat destruction campaign was begun in and around the infected area. Unfortunately only a small percentage of those killed were bacteriologically examined and no further plague infection of rats was discovered. The campaign was however kept up with varying success. By the offering of rewards a fair bag, reaching as many as 100 rats of sorts daily, was collected in and around the infected area, though many of them had been killed in far away parts of the town.

Owing to the imposition of restrictions on the liberty of the people by the drawing of a cordon round well wide of the infected area, and requiring persons desirous of leaving the area to take out passes or passports, their co-operation ceased. The rat bag dwindled to insignificant proportions in spite of the doubling of the reward.

Until 30th March, 1905, no plague cases were discovered, and as we had now been 33 days clear and no infected rats had been found, we naturally began to feel hopeful. But on 1st April a suspicious case was discovered in a village several hundred yards north of the originally infected village No. 1. This case, however, was afterwards pronounced to be probably filariasis, and nothing of a suspicious nature was ever afterwards discovered in the village, although no special measures except rat destruction were carried out therein.

On 21st April a case of plague was discovered in Handcock Parcherry, a pariah village about three-quarters of a mile from and south of villages Nos. 1, 2 and 3; to reach this Parcherry a large fishing village, No. 4, and another christian Parcherry, No. 5, were passed over or seemingly so. Three more cases of plague occurred in Handcock Parcherry between 21st April and 8th May. A short distance from the Parcherry half a dozen huts were erected, and every 24 hours six families were moved into these whilst their own huts were being disinfected. Evacuation of their own huts for a night was strictly insisted upon, and during this night commonsense rat exterminator was laid in each of the evacuated huts,



resulting in a fair total bag. Two more cases of plague occurred in this Parcherry on 26th June, the patients being visitors from a country village who were said to have arrived only three days previous to their attack. No cases have occurred in this village since and no infected rats have been found, though of late a large number have been killed here and all examined.

On 5th May a case was discovered in village No. 5, and two days afterwards this patient's wife developed the disease. On 11th June another case was discovered in this village and no cases have occurred since. The village was evacuated, but thanks to the intelligence and energy of the headman, rat destruction or attempted rat destruction has from the first been carried out here with vigour and is still kept up.

Village No. 4, known as Cassimode Kuppam, had long been under suspicion. The inhabitants—harbour coolies and fishermen—are a particularly illiterate and ignorant lot. Illness of every description was most carefully concealed, and rat catching in their village they would neither do themselves nor permit others to do. Several suspicious deaths had occurred, but no buboes were discovered on the corpses. On 20th May a female of the village was found suffering from fever and was removed for observation to the health camp. The following day she developed pneumonia, and microscopical examination of the sputa revealed abundant plague bacilli. Eight cases of plague occurred here between this and 18th August. The village was dealt with as Handcock Parcherry had been, but with the inhabitants opposing, concealing and obstructing at every step. However, no plague cases were discovered during the next four months and the Special Plague Staff was dispensed with. Again we had occasion to feel hopeful, though the fact that we could get no rats caught in village No. 4 left us in the dark with regard to what might be going on amongst them. The rats had certainly not been cleared out of this village, and it was reasonable to suppose that plague infection amongst them (if they had ever been infected) had not died out. The village was most carefully watched, and on 18th December, 1905 (after an interval of four months), a suspicious death was reported. On examination of the corpse an inguinal bubo was discovered. In this village, in January 20 cases occurred, in February 36, and up to 16th March last four more cases occurred in this and the blocks of huts between villages Nos. 4 and 5.

Rat destruction, which had practically ceased with the abolition of the Special Plague Staff, was renewed with vigour in and around the infected area from 18th December. From every rat caught, except those in an advanced state of decomposition, a spleen smear was made and microscopically examined. The first infected rat in this campaign was found on 29th December, 1905, in the area between villages Nos. 4 and 5. In the month of January 21 infected rats were found. 22 infected rats were found in February and two in March on the 10th of the month. All from an area which corresponded exactly—to an inch with the area in which human plague cases occurred. Since the 10th of March no infected rats have been found, and since 16th March there have been no plague cases amongst the people.

Altogether from 18th December to 18th April, 1888 rats were caught in and around the infected area, many having been brought from considerable distances outside, and 46 were found infected.

The evacuation of village No. 4 was begun in December, as it was hopeless attempting to clear the rats out of it while the people remained; commonsense rat poison was laid in each hut for several nights after evacuation, and the total given above does not nearly represent the total of rats killed by the poison or which died perhaps of plague, as on shifting the roofs of the huts numbers of dried up rat carcasses were found under the tiles or leaves.

With the evacuation of the village it was feared that though we were doing the best for the people of this particular village we would almost certainly drive the rats that escaped poisoning and with them the infection to adjacent villages, and this is what actually occurred to a limited extent. The infection gradually spread westwards until brought up by village No. 5, in which, as I have said, the people willingly co-operated with us in our campaign against rats.

Re-occupation of the evacuated area is now being gradually carried out, and by 1st May it is hoped all the people may be permitted to return to their permanent abodes, when their state of health will for several months be anxiously watched.

Since the close of this epidemic, no indigenous plague has been known to occur in rats or men in Madras City.

### 3. *The climate of Madras City.*

The climate of Madras City does not appear to offer any definite obstruction to the prevalence of plague. The following table gives the mean monthly temperature and humidity and, for comparison, the corresponding figures for three heavily infected towns.

TABLE I.

	Madras City		Bangalore		Belgaum		Bombay	
	Temperature	Humidity	Temperature	Humidity	Temperature	Humidity	Temperature	Humidity
January	75·1° F.	73 %	<b>68·5° F.</b>	<b>78 %</b>	69·9° F.	65 %	<b>73·0° F.</b>	<b>71 %</b>
February	76·7	73	<b>72·7</b>	<b>70</b>	72·8	59	<b>74·8</b>	<b>73</b>
March	80·0	74	<b>77·7</b>	<b>66</b>	77·1	55	<b>79·8</b>	<b>71</b>
April	84·0	74	81·2	72	79·2	57	<b>82·2</b>	<b>74</b>
May	86·7	67	80·2	76	77·7	66	86·2	72
June	86·4	62	75·8	81	72·6	80	83·8	82
July	84·5	65	73·8	84	70·1	91	80·2	88
August	83·3	70	73·7	85	<b>69·5</b>	<b>91</b>	80·8	85
September	83·0	72	<b>73·6</b>	<b>86</b>	<b>70·3</b>	<b>83</b>	79·5	86
October	80·6	78	<b>73·3</b>	<b>83</b>	<b>72·3</b>	<b>73</b>	80·6	80
November	77·5	79	<b>70·6</b>	<b>78</b>	<b>70·8</b>	<b>63</b>	79·5	70
December	75·5	77	<b>68·4</b>	<b>78</b>	<b>69·4</b>	<b>59</b>	76·1	76

The heavy type indicates the ordinary months of epidemic plague.

These figures show that though the summer months are hot and dry (cf. vol. VIII. p. 279), the conditions appear to be not unfavourable for plague from November to February.

4. *The rats of Madras.*

For several years past the Corporation of Madras City have undertaken rat destruction on a small scale. The methods they were adopting when the Commission started their observations in November, 1909, consisted in offering a reward of three pies (one farthing) for each rat brought in. Since this method of rat destruction was commenced in February, 1906, 1,220,686 rats have been destroyed. A staff of twenty-five peons were employed at collecting stations scattered at various places throughout the city whose duty it was to pay out each morning the rewards for rats received, affix a label to each rat on which was written the address of the house in which the rat was caught, and forward the rats so obtained to a central depot, where an Assistant Surgeon in charge of the operations checked the number of rats so brought in, and made notes of the species. All the rats received at the depot were dead and often in an advanced stage of decomposition. Most of the rats were caught with spring traps or had been killed with sticks, the bandicoots being almost exclusively obtained in the latter way. Very few rats were caught in ordinary wire or wooden traps. An examination of the methods employed demonstrated that little reliance could be placed on the accuracy of the addresses affixed to each rat. It was obvious that such methods, though they gave an idea of the species and relative numbers of the different species of rats that were present in the city, did not give us accurate enough information of the species of rats that frequented the houses of Madras, and this information was to us of prime importance.

Arrangements were therefore made for systematic trapping of portions of the city. In selecting sections of the city for our observations, we were guided more by the density of the human population than the presence of other factors favourable or otherwise to rat infestation. In other words we paid special attention to those portions of the town in which plague, if it ever did come to Madras, would be most likely to give rise to most serious difficulty and trouble. We also paid attention to those portions of the town in the near vicinity of the docks and goods depots of the railway lines, for it is in these localities that one would expect an epizootic to start if ever the city became infected. Our methods varied but little from those we have adopted in other towns, which have been described at length

in previous volumes of our reports. The trapping peons were in charge of an overseer, and a Sanitary Inspector was placed in charge of all the trapping operations.

Careful note was made as heretofore of the addresses of all houses trapped and those in which rats were caught. A daily examination was made of the rats caught and the fleas that they harboured. The results of our trapping and rat examination are set forth in tables II and III.

TABLE II. *Showing the number of rats caught in monthly periods.*

	<i>rattus</i>	Mice	Musk rats	Bandicoots	Total	Traps set	Rats per 100 traps	<i>rattus</i> per 100 traps
December 1909	1939	1386	129	28	3482	4949	67	<b>39</b>
January 1910	1965	1369	95	34	3463	4660	74	<b>42</b>
February	1741	1457	113	33	3344	4680	71	<b>37</b>
March	1236	1445	96	11	2788	3470	80	<b>36</b>
April	1059	1196	81	7	2343	3288	71	<b>32</b>
May	1437	1337	133	34	2941	4856	61	<b>30</b>
June	1378	1276	114	38	2806	5200	54	<b>26</b>
July	1302	1107	86	34	2529	5200	49	<b>25</b>
August	1391	1435	87	28	2941	5718	51	<b>24</b>
September	1593	1355	74	32	3054	5855	52	<b>27</b>
October	1389	1488	123	37	3037	5280	58	<b>26</b>
November	1233	1638	122	23	3016	5415	56	<b>23</b>
Total	17,663	16,489	1,253	339	35,744	58,571	61.0	<b>30.2</b>
	49.4 %	46.1 %	3.5 %	1.0 %	100 %			

TABLE III. *Showing the data regarding the breeding of *Mus rattus*.*

	<i>rattus</i> dissected	Adult females	Pregnant females	Percentage of adult females pregnant	Average number of foetuses per pregnant female	Young rats	Percentage of young rats
December 1909	1694	653	188	<b>28.8</b>	5.3	588	34.8
January 1910	1573	611	181	<b>29.6</b>	5.3	498	31.7
February	1246	482	172	<b>35.6</b>	5.5	419	33.6
March	1166	442	134	<b>30.3</b>	5.1	458	<b>39.3</b>
April	781	333	103	<b>30.9</b>	5.4	274	35.0
May	692	259	59	22.8	5.0	235	33.9
June	877	347	96	27.6	5.1	303	34.6
July	688	207	54	26.1	5.0	251	<b>36.5</b>
August	797	269	56	20.8	5.1	312	<b>39.1</b>
September	835	291	75	25.7	5.3	337	<b>40.3</b>
October	837	294	75	25.6	5.2	281	33.7
November	766	274	65	23.8	5.3	281	<b>36.8</b>
Total	11,952	4,462	1,258	28.2	5.2	4,237	35.4

A table is also given (table IV) which shows the number and species of rats received at the Central Municipal rat collecting station. A comparison of the table that gives the number of rats of different species brought into the Municipal depot with the table that records the result of our trapping operations for the same months, is of some interest. In the case of trapping, it will be seen that *Mus rattus* formed 49·4 % of the total catch. Of the Municipal rats brought in dead, 51 % were *Mus rattus*. When we consider the other species, a remarkable difference occurs in their

TABLE IV. *Rats brought into the Municipal rat collecting depot.*

	Bandicoots	Bandicoots per cent.	<i>Mus rattus</i>	<i>Mus rattus</i> per cent.	Mice	Mice per cent.	Musk rats	Musk rats per cent.	Other species	Other species per cent.	
Dec. 1909	4627	17·5	15062	57·0	6026	22·4	650	2·5	60	0·2	26425
Jan. 1910	4369	18·9	12706	54·9	5516	23·8	544	2·4	2	0·008	23137
Feb.	4201	21·5	10802	55·3	4099	21·0	423	2·2	7	0·03	19532
Mar.	4345	21·2	11847	57·7	3897	19·0	423	2·1	21	0·1	20533
Apr.	3895	26·2	7210	48·5	3345	22·5	380	2·6	35	0·2	14865
May	3666	27·9	6236	47·4	2873	21·8	357	2·7	31	0·2	13163
June	5424	29·5	8210	44·7	4080	22·2	633	3·4	40	0·2	18387
July	5338	26·1	10039	49·1	4556	22·3	457	2·2	40	0·2	20430
Aug.	4207	22·2	10585	55·8	3728	19·6	425	2·2	28	0·1	18973
Sept.	4379	22·4	9522	48·8	4708	24·1	880	4·5	39	0·2	19528
Oct.	6819	29·0	9993	42·5	5623	23·9	1019	4·3	50	0·2	23504
Nov.	7667	30·2	12076	47·1	5180	20·4	453	1·8	26	0·1	25402
Total	58937	24·3	124288	51·0	53631	22·0	6644	2·7	379	0·8	243879

relative frequency as recorded in the two tables. Of the rats brought in dead 24·3 % were bandicoots. Of rats caught in traps set in houses only 1·0 % were bandicoots. This difference is very readily explained. It was mentioned above that many of the rats brought into the Municipal depots were killed with sticks. The bandicoots that inhabit the open gullies that border both sides of the road in the native quarters of the town can be seen any night scampering about the roads in large numbers. They offer a much larger mark than other species of rodent, and what is more they are surprisingly easily killed with a blow. The natives have told us that a single blow is always sufficient to kill a bandicoot and that a *rattus* requires several to disable him. In the second place, the rat traps that we employed were not large enough to capture a full-grown bandicoot though a half-grown animal could have readily entered. In spite of this second

fact, it was only on extremely rare occasions that a young bandicoot was caught in our traps. The inhabitants of the native city have frequently assured us that bandicoots enter the houses at night in numbers and that the inhabitants are frequently disturbed at night by the noise made by these large and clumsy rodents. It is strange, therefore, that we were not successful in capturing more young ones. Our traps were always placed inside the houses. We are compelled to believe that the invasion of dwelling houses with bandicoots (at any rate young ones) is an uncommon event<sup>1</sup>.

The absence of *decumanus* from Madras is striking. The presence of the bandicoot in such large numbers has been offered as an explanation of this fact. It is difficult to believe that people who have propounded such a theory can have made a study of the habits of the bandicoot. They have endowed the bandicoot with ferocious qualities of which it is quite innocent. We have had *Mus rattus*—a very much less formidable rat than *Mus decumanus*—living in cages with bandicoots for long periods. In no instance were the *Mus rattus* molested.

Mice form 46.1 % of the total number of rodents that were caught in traps, whereas only 22 % of those brought in dead to the Municipal depots were mice. This difference is readily accounted for when the difference in the manner in which they were obtained is taken into consideration. Both sets of figures demonstrate how extremely numerous mice are in Madras, much more so than in other places where the Commission have undertaken similar observations.

The most important fact that the two tables bring out is that in Madras, as in the vast majority of Indian towns, *Mus rattus* is the predominant house rat. As far as we are able to judge from our trapping results, the infestation of Madras City with *Mus rattus* is not definitely less than in other places where we have worked which have been plague infected. Thus the average number of *rattus* per 100 traps set was 30; with similar methods of counting, except that in Madras the places where traps were set were to some extent selected (above, p. 213), in Poona the figure was 28, in Belgaum 23, in Parel 24. On the other hand in various places in Eastern Bengal and Assam, which were free from plague, from two to ten rats were caught (vol. XI. supplement, p. 187). It should perhaps, however, be noted that in Poona, after a year without any plague, the *rattus* per 100 traps rose to 42 and after two years to as many as 70. The great relative abundance of mice in Madras also suggests that the rat

<sup>1</sup> In Belgaum however bandicoots live in the houses: vol. x. p. 459.

population is not really so dense as in the other places mentioned, where hardly any mice were caught.

The *susceptibility of Madras rattus to plague* has been examined on many occasions. The details are given elsewhere (below, p. 257). The result is that Madras *rattus* are exceptionally susceptible to the hypodermic injection of small doses of an emulsion of a plague rat's spleen, 95 to 100 % dying of plague as against 20 to 40 % of *rattus* from Bombay, Poona or Belgaum.

### 5. The rat fleas of Madras City.

Practically the only species of flea found on the rats in Madras City is *X. cheopis*. On one or two occasions a specimen of *C. felis* was obtained, but such an occurrence was very rare.

Table V shows the result of the flea counts carried on throughout a complete year.

TABLE V. *Showing the prevalence of fleas with meteorological data.*

	Number of <i>rattus</i> "flea counted"	Fleas caught	Fleas per <i>rattus</i>	Mean temperature	Mean humidity
December, 1909	1663	8726	5·2	77·8	76
January, 1910	1573	8429	5·4	76·5	76
February	1252	5340	4·3	78·0	73
March	1164	4417	3·8	80·0	76
April	781	2626	3·4	85·1	76
May	692	2713	3·9	88·5	65
June	877	1913	2·2	86·4	65
July	688	1790	2·6	84·3	74
August	797	2850	3·6	84·7	76
September	835	3259	3·9	83·1	77
October	809	2905	3·6	81·5	81
November	766	3256	4·3	76·6	81
Total	11,897	48,224	4·05		

The figures show that in Madras as elsewhere there is a seasonal variation in the abundance of rat fleas. *X. cheopis* was most abundant in December and January while only about half as many were found in the hot dry weather in June and July. The flea prevalence and range of seasonal variation is very similar to what was observed for *rattus* in Bombay (vol. VIII. p. 297), the maximum in Madras falling some two months earlier. But in other, and plague infected, places which have been investigated by us, though the minimum figure is about the same (two or three per rat), during the earlier part of the plague season each *rattus* has yielded on the average 19 fleas in Belgaum, 12 in Dhand, 13 in Kasel, 9 in Poona (vol. XI. supplement, p. 188), 13 in Lucknow, 18 in Cawnpore<sup>1</sup>, as against a monthly

<sup>1</sup> The details will appear in a later report.

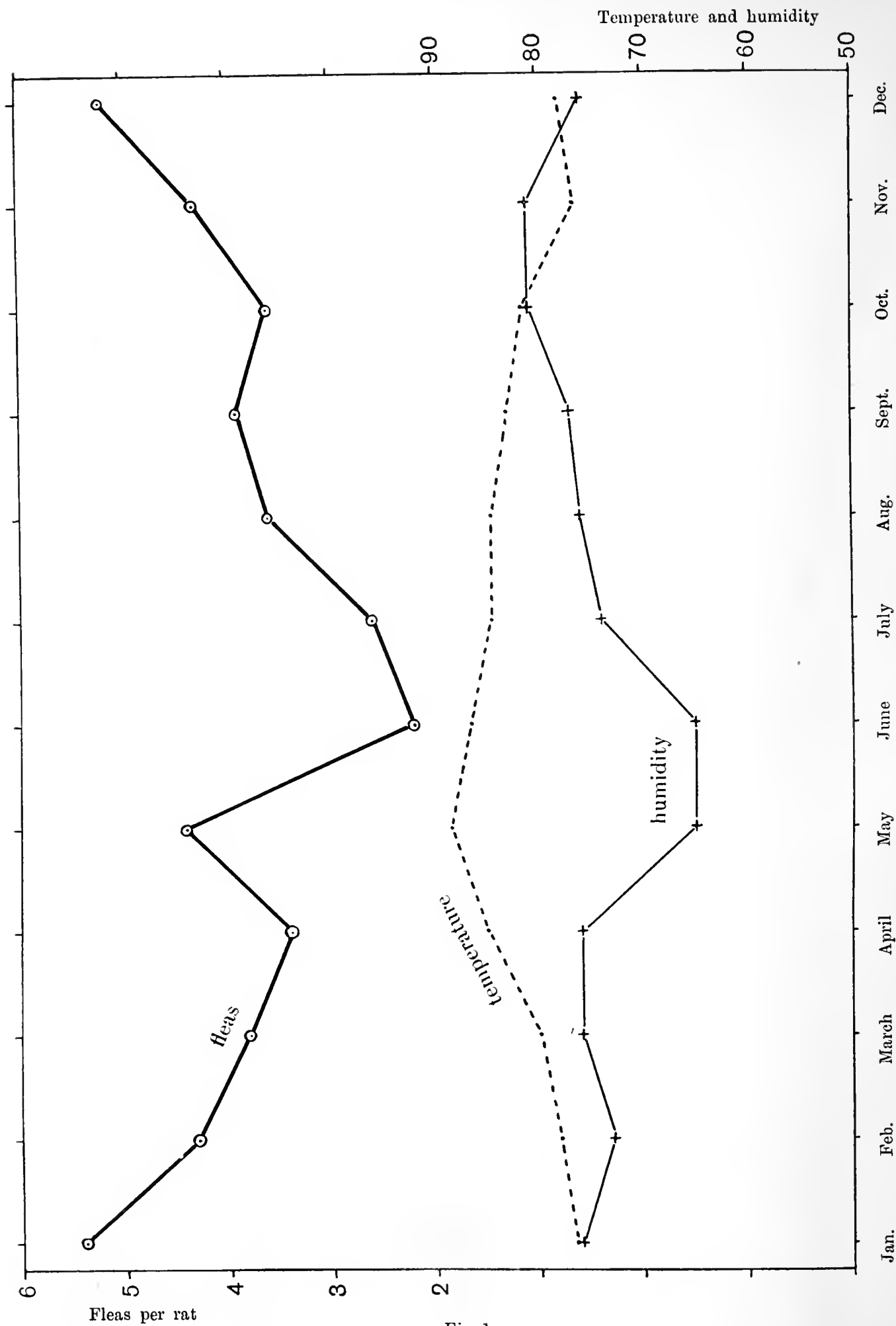


Fig. 1.



maximum of 5 in Madras City. In Bombay, it should also be noted, on *decumanus* the fleas rise to as many as 14 per rat. In view, however, of the Bombay figures for *rattus*, it cannot be considered that the relative paucity of fleas in Madras constitutes a necessarily effective obstacle to the establishment and prevalence of plague infection.

The mice of Madras harbour relatively few fleas. On 1621 mice examined when fleas were most numerous on *rattus*, only 241 fleas were obtained, an average of one flea on seven mice (cf. vol. x. p. 526).

#### 6. *Summary and conclusions.*

As the result of our enquiries we find therefore that the conditions of house construction in Madras City are not unfavourable to the establishment of epidemic plague, and that suitable climatic conditions prevail during the winter months. As regards the rats and rat fleas, it appears that, though neither are so plentiful as in other plague-infected places investigated by us, there are probably enough of both to maintain plague, though perhaps few enough to render implantation difficult. The rats, moreover, are exceptionally susceptible to plague. It appears, therefore, that Madras City is not immune to plague in the sense that the conditions prevailing there (as far as they have been investigated by us) are such that plague could not become established in the place. There has been no large outbreak which definitely proves this, for the single epidemic from which Madras has suffered was of a mild character. On the other hand infection was protracted and, as will have been gathered from the description given above, the outbreak was confined to a few small hamlets on the outskirts of the town and was dealt with with the most exemplary vigour and determination by the sanitary authorities, and the fact that the human mortality was relatively slight cannot under the circumstances be taken as evidence that the city is naturally an unsuitable place for plague.

The circumstances which condition the successful implantation of imported plague are probably the same as those which determine the prevalence of infection when once established. Imported plague is for example most likely to start an epidemic if it reaches a place at a time when meteorological conditions are most favourable and rat fleas most numerous. In Madras such circumstances are most favourable at a time of year (December to February) which coincides with the plague season in such a possible source of infection as Bangalore.

It seems then likely that Madras has escaped from plague because infection has been unable to reach there or has met with some

obstruction immediately on its arrival. The possible sources from which infection might have been brought are very large, considering the abundant traffic with the infected areas in Mysore and in Bombay presidency. Assuming that infection travels about the country mostly in infected fleas associated either with such merchandise as grain or with the persons of human beings, it is clear that the likelihood of infection traversing any given distance will be proportional to the conditions being favourable to the life of the flea. Experiments have shown (see below, p. 317) that meteorological circumstances have a very large influence on the duration of life of rat fleas apart from their host, a cool moist atmosphere allowing them to survive for ten times as long as in hot dry air. Evidently therefore fleas would have some difficulty in arriving alive at any place which was surrounded by a zone of country where a high temperature, especially in conjunction with a low humidity, continuously prevailed. Madras is on the whole a hot place, but in the cooler months the temperature would apparently allow fleas to live for some considerable time though there is no definitely cold weather to afford really favourable conditions for flea importation.

So much for the influence of natural circumstances. Artificial influences are found in the passport system and its associated energetic sanitary administration<sup>1</sup>. As is often the case with practical sanitation where mixed experiments are made by taking various precautionary measures simultaneously, it is difficult to form an accurate estimate of the precise effect of the passport system in vogue in the province of Madras. It does not seem to discover very many cases of peripatetic plague and a fair number of infected persons appear to escape its surveillance. On the other hand its influence in preventing persons who are or may be infected from starting on any journey is very likely considerable.

In view of the above results it seemed proper to extend the scope of the enquiry to other parts of the Madras Presidency, especially as regards the conditions which have a bearing on the facilities for the importation of infection. This investigation is now being undertaken and will shortly be completed, and we reserve any further discussion of the questions outlined above until the whole problem of plague in the Madras Presidency can be examined in detail.

<sup>1</sup> See on the whole question, and especially on this subject, the important papers by Col. W. G. King, C.I.E., late Sanitary Commissioner to the Government of Madras, on "The Prevention of Plague in the Madras Presidency" in the *Journal of State Medicine*, 1912.

XLIX. STATISTICS OF THE OCCURRENCE OF PLAGUE  
IN MAN AND RATS IN BOMBAY, 1907—1911.

SINCE the Commission brought their enquiry into rat plague in Bombay to an end in the latter part of 1906, the collection of rats on a large scale has been continued by the Bombay City Municipal Health Department and these animals have been examined for plague at the Bombay Bacteriological Laboratory where the headquarters of the Commission is established. The figures have been published from time to time in the annual reports of the Bacteriological Laboratory by the Director of the Laboratory and in the tables below they are collected together on a uniform plan.

The data for human plague are taken from the returns of the Health Department. The rat figures have been corrected for the fact that in some weeks the collection and examination of rats was suspended for one or two days.

The results are of much interest showing as they do that the phenomena (vol. VII. pp. 759, 764 and chart) observed in 1905-6 have since been repeated year after year in Bombay City. More particularly the figures show how the rat epidemic precedes the human epidemic and how the epidemic among *M. decumanus* comes a little before that in *M. rattus*.

1907.

*Total rats examined 107,875.*

		Human plague mortality		Plague infected <i>rattus</i>		Plague infected <i>decumanus</i>	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	63	- 76	108	- 43	691	+12
„	16—31	108	- 59	245	+29	1198	+94
February	1—15	211	- 21	331	+75	1412	+129
„	16—28	393	+46	485	+155	1798	+191
March	1—15	979	+264	602	+217	2093	+239
„	16—31	1455	+441	764	+303	2403	+289
April	1—15	1190	+342	491	+159	1441	+133
„	16—30	794	+195	331	+75	867	+46
May	1—15	443	+65	159	- 16	443	- 28
„	16—31	214	- 20	88	- 53	265	- 57
June	1—15	77	- 71	20	- 91	104	- 83
„	16—30	46	- 83	40	- 79	79	- 87
July	1—15	42	- 84	33	- 82	98	- 84
„	16—31	28	- 90	18	- 90	69	- 89
August	1—15	20	- 93	47	- 75	107	- 82
„	16—31	50	- 82	67	- 65	157	- 74
September	1—15	51	- 81	79	- 58	143	- 76
„	16—30	63	- 76	111	- 42	144	- 76
October	1—15	59	- 78	135	- 29	160	- 74
„	16—31	43	- 84	159	- 16	174	- 72
November	1—15	43	- 84	96	- 49	249	- 59
„	16—30	29	- 89	66	- 65	209	- 66
December	1—15	26	- 90	34	- 82	200	- 67
„	16—31	28	- 90	44	- 77	299	- 51
Total		6,455		4,553		14,803	

1908.

Total rats examined 110,512.

		Human plague mortality		Plague infected <i>rattus</i>		Plague infected <i>decumanus</i>	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	20	- 91	57	- 59	410	- 28
"	16—31	59	- 74	86	- 26	656	+16
February	1—15	92	- 59	142	+22	796	+39
"	16—28	264	+16	166	+43	1217	+113
March	1—15	568	+150	256	+120	1675	+193
"	16—31	914	+302	321	+176	2273	+298
April	1—15	942	+315	353	+204	1885	+230
"	16—30	907	+299	308	+164	1336	+134
May	1—15	626	+176	221	+90	886	+55
"	16—31	340	+50	143	+23	475	- 17
June	1—15	144	- 36	84	- 28	211	- 63
"	16—30	62	- 73	55	- 54	189	- 67
July	1—15	62	- 73	58	- 59	116	- 80
"	16—31	74	- 67	71	- 39	129	- 77
August	1—15	77	- 66	91	- 22	144	- 75
"	16—31	65	- 71	60	- 49	153	- 73
September	1—15	52	- 77	35	- 70	167	- 70
"	16—30	55	- 76	59	- 49	131	- 77
October	1—15	32	- 86	46	- 69	120	- 79
"	16—31	32	- 86	55	- 53	96	- 83
November	1—15	19	- 91	38	- 67	115	- 80
"	16—30	11	- 95	32	- 72	129	- 77
December	1—15	15	- 93	29	- 75	182	- 68
"	16—31	9	- 96	23	- 80	221	- 61
Total		5,441		2,789		13,512	

1909.

*Total rats examined 91,540.*

		Human plague mortality		Plague infected <i>rattus</i>		Plague infected <i>decumanus</i>	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	24	- 89	46	- 62	244	- 39
„	16—31	42	- 80	74	- 22	362	- 10
February	1—15	68	- 60	103	<b>+ 8</b>	504	<b>+ 25</b>
„	16—28	209	- 4	151	<b>+ 59</b>	766	<b>+ 90</b>
March	1—15	481	<b>+ 121</b>	231	<b>+ 144</b>	1199	<b>+ 198</b>
„	16—31	1013	<b>+ 366</b>	336	<b>+ 254</b>	1599	<b>+ 298</b>
April	1—15	810	<b>+ 273</b>	270	<b>+ 185</b>	1206	<b>+ 200</b>
„	16—30	994	<b>+ 358</b>	273	<b>+ 188</b>	976	<b>+ 143</b>
May	1—15	663	<b>+ 206</b>	159	<b>+ 68</b>	564	<b>+ 40</b>
„	16—31	329	<b>+ 52</b>	84	- 12	308	- 23
June	1—15	103	- 53	30	- 68	133	- 67
„	16—30	56	- 74	29	- 70	110	- 73
July	1—15	48	- 78	20	- 79	104	- 74
„	16—31	57	- 74	29	- 70	101	- 75
August	1—15	69	- 68	28	- 71	94	- 77
„	16—31	69	- 68	41	- 57	75	- 81
September	1—15	38	- 82	37	- 61	95	- 76
„	16—30	40	- 81	22	- 76	80	- 80
October	1—15	25	- 88	32	- 66	111	- 72
„	16—31	20	- 90	43	- 55	143	- 64
November	1—15	13	- 94	37	- 60	159	- 60
„	16—30	13	- 94	64	- 33	188	- 53
December	1—15	12	- 94	62	- 34	227	- 43
„	16—31	11	- 95	76	- 19	297	- 26
Total		5,207		2,277		9,645	

1910.

Total rats examined 119,456.

		Human plague mortality		Plague infected <i>rattus</i>		Plague infected <i>decumanus</i>	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	22	- 85	96	- 22	382	- 16
	,, 16—31	61	- 60	179	<b>+46</b>	585	<b>+28</b>
February	1—15	126	- 17	207	<b>+68</b>	824	<b>+80</b>
	,, 16—28	169	<b>+11</b>	246	<b>+100</b>	1008	<b>+121</b>
March	1—15	259	<b>+70</b>	194	<b>+58</b>	1005	<b>+120</b>
	,, 16—31	439	<b>+189</b>	195	<b>+58</b>	1051	<b>+130</b>
April	1—15	442	<b>+190</b>	205	<b>+67</b>	1065	<b>+133</b>
	,, 16—30	482	<b>+217</b>	231	<b>+88</b>	1033	<b>+126</b>
May	1—15	497	<b>+227</b>	181	<b>+48</b>	741	<b>+62</b>
	,, 16—31	342	<b>+125</b>	102	- 17	442	- 3
June	1—15	168	<b>+10</b>	87	- 29	321	- 30
	,, 16—30	111	- 27	68	- 45	276	- 40
July	1—15	102	- 33	96	- 22	279	- 39
	,, 16—31	120	- 21	75	- 39	217	- 53
August	1—15	68	- 55	65	- 47	193	- 58
	,, 16—31	65	- 57	105	- 14	213	- 53
September	1—15	25	- 83	69	- 43	157	- 66
	,, 16—30	27	- 82	87	- 29	141	- 69
October	1—15	33	- 78	82	- 33	90	- 80
	,, 16—31	27	- 82	107	- 13	148	- 68
November	1—15	27	- 82	81	- 34	134	- 70
	,, 16—30	17	- 89	74	- 40	150	- 67
December	1—15	14	- 90	58	- 55	175	- 62
	,, 16—31	18	- 88	46	- 63	221	- 51
Total		3,661		2,936		10,851	

1911.

*Total rats examined, 134,401.*

		Human plague mortality		Plague infected <i>rattus</i>		Plague infected <i>decumanus</i>	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	25	- 85	59	- 42	337	- 32
„	16—31	85	- 49	109	+ 8	530	+ 8
February	1—15	129	- 22	155	+ 54	786	+ 60
„	16—28	251	+ 51	204	+ 102	1140	+ 132
March	1—15	366	+ 121	184	+ 82	1336	+ 172
„	16—31	497	+ 199	242	+ 140	1600	+ 225
April	1—15	686	+ 313	267	+ 165	1533	+ 212
„	16—30	612	+ 269	223	+ 120	1049	+ 113
May	1—15	507	+ 205	245	+ 142	872	+ 77
„	16—31	300	+ 81	120	+ 19	517	+ 5
June	1—15	98	- 41	56	- 44	266	- 46
„	16—30	97	- 42	41	- 59	228	- 54
July	1—15	54	- 67	36	- 65	156	- 68
„	16—31	58	- 65	49	- 51	167	- 66
August	1—15	22	- 87	41	- 59	137	- 72
„	16—31	42	- 75	19	- 81	113	- 77
September	1—15	27	- 84	64	- 37	114	- 77
„	16—30	33	- 80	47	- 53	131	- 73
October	1—15	21	- 87	55	- 46	129	- 74
„	16—31	12	- 93	43	- 57	99	- 80
November	1—15	13	- 92	43	- 58	106	- 78
„	16—30	16	- 90	43	- 58	139	- 72
December	1—15	11	- 93	38	- 63	127	- 74
„	16—31	30	- 82	43	- 58	191	- 61
Total		3,992		2,436		11,803	

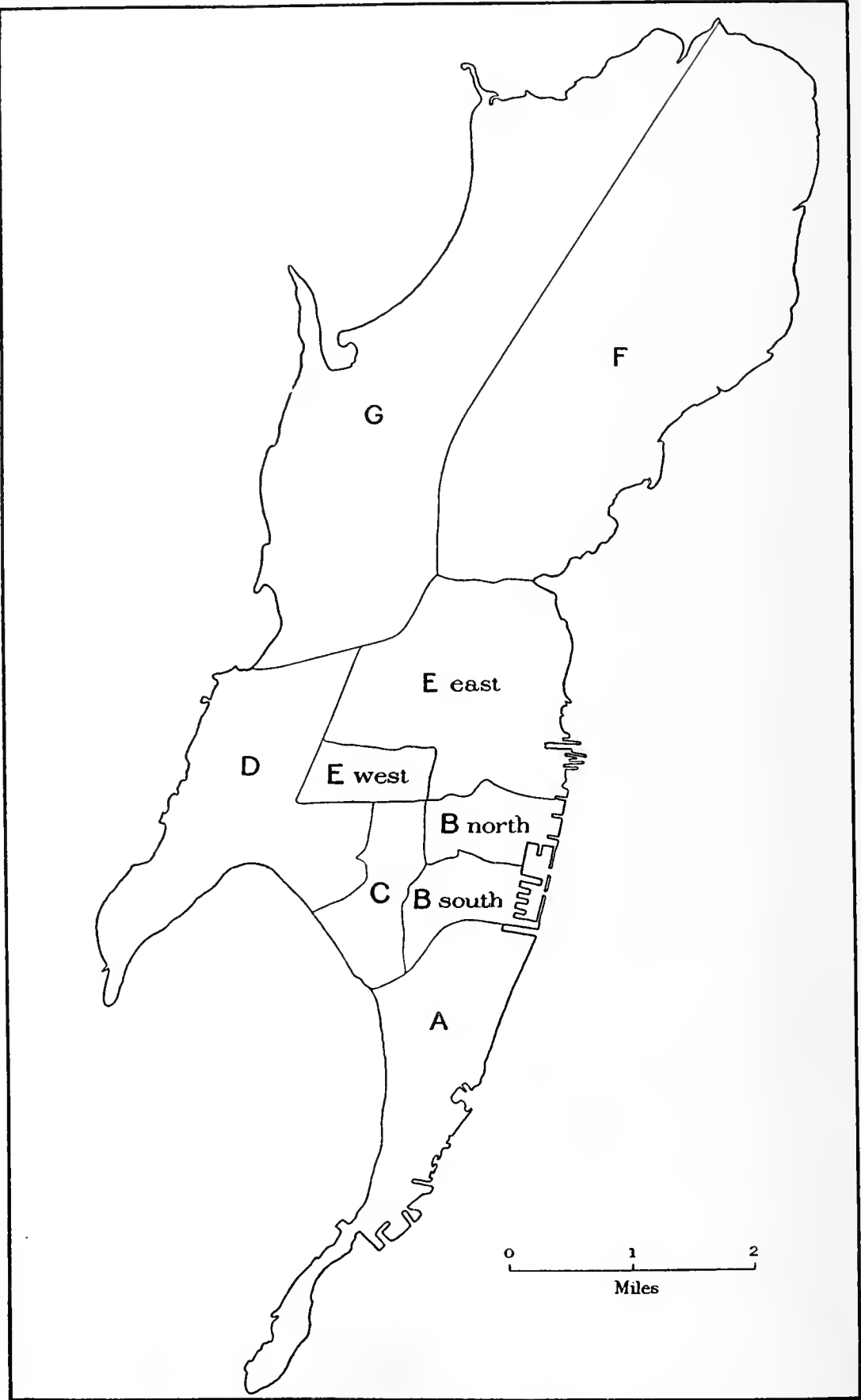


# L. THE DISTRIBUTION OF WHITE-BELLIED *MUS RATTUS* IN BOMBAY ISLAND.

WE have previously noted the occurrence in small numbers of the white-bellied form of *Mus rattus* known as *M. alexandrinus* in Poona (vol. x. p. 521) and their occurrence in Bombay has been known since we began our observations there (vol. vii. p. 743). In the present note we give statistics showing that this form is not evenly distributed about the island, and is relatively more abundant where there is plenty of open ground. The table gives the rat figures, all the animals having been trapped alive in houses and buildings, together with the density of the population in the different wards<sup>1</sup> as being the most convenient expression of the conditions which seem to influence the frequency of *alexandrinus*.

Ward	A			B north			B south			C			D		
Month	Total <i>rattus</i>	<i>Alexandrinus</i>	Per mille	Total <i>rattus</i>	<i>Alexandrinus</i>	Per mille	Total <i>rattus</i>	<i>Alexandrinus</i>	Per mille	Total <i>rattus</i>	<i>Alexandrinus</i>	Per mille	Total <i>rattus</i>	<i>Alexandrinus</i>	Per mille
Jan. 1910	3621	16	4	5211	9	2	4792	31	6	5374	4	1	3900	8	2
February	3007	7	2	4607	11	2	4391	49	11	4225	1	0	3431	6	2
March	3174	29	9	4152	13	3	4461	30	7	3565	3	1	3551	8	2
April	2635	9	2	3788	3	1	4457	49	11	2740	1	0	2734	4	1
May	2147	10	4	3300	15	5	3711	21	6	2100	1	0	2422	4	2
June	2238	22	9	3873	12	3	3664	34	9	2267	7	3	2539	2	1
July	2344	16	6	3968	17	4	3851	44	11	2982	6	2	2394	9	4
August	2840	8	2	4007	9	2	4502	35	8	2947	4	1	2904	13	4
September	2872	19	6	3326	13	4	4589	32	7	2681	3	1	4286	3	1
October	2860	12	4	3567	4	1	6248	85	14	2749	5	2	4141	3	1
November	3097	19	6	4615	5	1	7019	62	9	3939	5	1	4953	10	2
<b>Total</b>	<b>30,835</b>	<b>167</b>	<b>5</b>	<b>44,414</b>	<b>111</b>	<b>2</b>	<b>51,685</b>	<b>472</b>	<b>9</b>	<b>35,569</b>	<b>40</b>	<b>1</b>	<b>37,255</b>	<b>70</b>	<b>2</b>
Population per acre	50			186			287			416			70		
Ward	E east			E west			F			G			Total		
Jan. 1910	4720	54	11	2593	4	2	3153	86	27	2171	50	23	35,535	262	7
February	4018	26	6	2192	2	1	2595	70	27	2119	43	20	30,585	215	7
March	3837	73	19	1966	0	0	2439	14	6	1664	48	29	28,809	218	8
April	3760	51	14	2000	0	0	2473	53	21	1701	36	21	26,288	206	8
May	3260	38	12	1823	0	0	1969	11	6	1122	33	29	21,854	133	6
June	3656	54	15	2550	4	2	1732	45	26	897	9	10	23,416	189	8
July	3369	53	16	3259	0	0	1633	52	32	1444	32	22	25,244	229	9
August	4262	96	23	3761	9	2	1674	72	43	1176	17	14	28,073	263	9
September	3869	48	12	3398	4	1	1987	53	27	1448	36	25	28,456	211	7
October	4013	97	24	2965	8	3	1996	89	45	1839	69	38	30,378	372	12
November	5180	76	15	3027	5	2	2392	68	28	2697	52	19	36,919	302	8
<b>Total</b>	<b>43,944</b>	<b>666</b>	<b>15</b>	<b>29,534</b>	<b>36</b>	<b>1</b>	<b>24,043</b>	<b>613</b>	<b>26</b>	<b>18,278</b>	<b>425</b>	<b>23</b>	<b>315,557</b>	<b>2600</b>	<b>8.2</b>
Population per acre	82			258			18			39					

<sup>1</sup> The grouping of sections into wards is not that which is now used by the Health Authority. For the sections see vol. vii. pp. 727, 778.



Map of Bombay Island.

## LI. THE IMMUNITY OF THE WILD RAT IN INDIA.

It is natural to suppose that the relative susceptibility or immunity of the rat to plague infection is a matter of considerable importance in the epidemiology of this disease. We have referred to the immunity of the rat to plague on several occasions, among others, for example, when discussing the factors which possibly tend to bring an epidemic of this disease to a close. We were not, however, able to bring forward any experimental evidence to show that the immunity of the rat was a factor of importance in regulating the course of an epidemic. We made several attempts to prove that rats possess a high degree of immunity at the close of an epizootic. In particular we may refer to an attempt to test this point which is mentioned in our first report on our Poona observations (vol. X. p. 523), and also to the results obtained when we tested the immunity of the survivors of the epizootics we produced in our godowns (vol. X. p. 332; this volume, p. 298).

In the Poona experiments we were confronted with great technical difficulties. We found that the varying climatic conditions at different times of the year influenced greatly the mortality among the rats under experiment; a cold night was sufficient to cause a high mortality, sick rats apparently dying from the effects of cold. Indeed we found as the result of this experiment that, in place of the immunity of Poona rats increasing as the epizootic progressed, their immunity to plague, as tested by our method of subcutaneous injection of small doses of plague bacilli, varied rather with the temperature, so that the colder the weather the greater was the mortality. Again, during the monsoon months, when the cages in which the rats were kept were liable to remain damp for days, many deaths among the rats occurred either from plague or from some other cause; it was impossible to say that plague had not been the cause of death for all the rats had been injected with living plague bacilli and we could generally isolate that organism from their tissues and organs. In short we learned that the circumstances and surroundings in which the rats were kept had a considerable influence in increasing or lowering the death rate among them. Again it was a matter of no small difficulty to find some means of regulating the dose of plague bacilli injected so as to maintain an even dose in a long series

of experiments; two variable factors had to be regulated—the virulence of the organism and the number used in each dose. We soon learned that broth cultures were of little use for our purpose, and after a prolonged series of trials of different methods we adopted the method of spleen emulsions to be described presently. We also learned that in order to obtain more or less consistent results it was necessary to inoculate an equal number of rats of constant and standard resistance at the same time as the rats whose immunity to plague we proposed to measure. These experiments therefore, dealing as they did with many hundreds of rats, although they failed to demonstrate any increase in immunity among the Poona rats at the close of an epizootic, served a most useful purpose, for the experience gained was of great use to us in the experiments presently to be described.

In the godown experiments referred to above, in which we tested the immunity of the survivors of epizootics and compared their immunity with that of the rats which survived in the control godowns, we were forced to the conclusion that it was probable that the apparently high degree of immunity of the rats which survived the epizootics as compared with those of the control godowns was due to the death of susceptible individuals during the epizootic rather than to any immunity acquired by the rats in having suffered and recovered from the disease. This observation, together with the fact that we had found the rats caught in Belgaum very resistant to doses of plague which we knew killed a fair proportion of Bombay rats (see vol. x. p. 458), raised the question, whether the rats in places which had suffered from plague were more resistant to the disease than those caught in places which had not been invaded by it. We were confronted with the problem, were the rats in plague-infected places immune to plague because they had suffered from the disease or was this immunity a racial immunity or due to some other cause?

About this time we were making an inquiry as to the reasons why the Madras Presidency had suffered less from plague than other parts of India and for this purpose we were collecting and examining rats caught in Madras City<sup>1</sup>, a place which had practically escaped the ravages of this disease. The occasion therefore afforded us an excellent opportunity to secure, as a standard of resistance, rats which we knew had not been exposed to infection. Arrangements were therefore made at our headquarters in Bombay to receive rats caught in Madras and Poona and to compare their immunity with that of rats caught in Bombay.

<sup>1</sup> See above, p. 209.

We decided to carry out the experiment in two ways:—(1) by inoculating a large number, if possible one hundred, rats at a time from each place with the same dose of plague bacilli subcutaneously, and (2) by subjecting equal numbers of rats from the three places to infection by fleas which had fed on rats infected by plague.

The technique adopted in the first experiment was as follows. One hundred rats from Madras and Bombay and seventy-six rats from Poona were placed in separate cages, two rats in each cage. The spleen of a rat which had died of acute plague having been removed aseptically was weighed. It was then placed in a sterile mortar and ground up with a known quantity of saline solution. The larger particles of this emulsion were allowed to settle and the supernatant fluid was decanted off; to this emulsion of bacilli fresh saline was added as necessary so that one cubic centimeter of the ultimate solution contained what was regarded as the equivalent of one one-hundredth part of a milligramme of the infected spleen<sup>1</sup>. This we found to be the most convenient dose to use in our experiments, but occasionally we used larger doses and this was effected by decreasing the amount of saline added to the emulsion. The cages containing the rats were kept in special godowns so that they were all subjected to the same conditions. The events which occurred in this experiment are shown below. In all, while 83 % of Madras rats died of plague (the remainder of the hundred having died of other causes than plague), only 44 % of Bombay rats and 30 % of Poona rats succumbed to the disease. Madras rats therefore were highly susceptible to the disease; Bombay and Poona rats comparatively immune.

In the second experiment we attempted to find out whether the above conclusion held true when the disease was propagated among the rats by infected fleas. For this purpose 25 Bombay rats were placed in each of two godowns, in two other godowns 25 Madras rats were placed in each, and in a third pair of godowns 25 Poona rats were placed in each. Into each of the six godowns containing the rats mentioned above five inoculated rats were introduced to infect the fleas which were present in the godown. In order to estimate the number of fleas in the godowns a weekly flea count was made; this was accomplished by counting the number of fleas on a certain number of the rats in each godown and finding the average number of fleas per rat. During the course of the experiment, as inoculated rats died off, others were added

<sup>1</sup> In later experiments the actual number of bacilli inoculated was ascertained by plating.

to the godowns so that five were added to each godown on the 28th January when the experiment started, five on the 12th February and three to each on the 26th February. Experiment II details the daily events occurring in the six godowns. Godowns 1 and 2 contained Bombay rats which showed an average weekly flea count of 4.2 and 2.2 respectively. Only three uninoculated rats died of plague in these godowns. Godowns 3 and 4 contained Poona rats. They showed an average weekly flea count of 4.4 and 4.7 fleas per rat respectively. Only one rat died in these godowns. Godowns 5 and 6 contained Madras rats. The flea count in these godowns was 4.3 and 5.0 respectively, and in them as many as thirty-six rats died of plague. This experiment showed, as the first had shown, that Poona and Bombay rats are, relatively to Madras rats, highly immune to infection.

In the first experiment a considerable mortality is recorded under the heading "deaths not due to plague." In judging whether a rat should be regarded as having died from plague or from other causes, no attempt was made to secure cultures, since all the animals alike had been inoculated, and we relied wholly on the naked-eye discovery of the typical post-mortem signs of plague with which we were now very familiar. The high mortality during the first few days was due partly to the rats having been recently trapped and so being unaccustomed to live in captivity and partly to the difficulty of handling wild rats for inoculation without causing them some injury. In other experiments the excess of "deaths not plague" occurred later on and was evidently not directly due to either of these factors. In our later experiments most of the early mortality was avoided by keeping the rats for a little while before inoculation and by greater technical experience; in the majority of experiments more than 100 rats were inoculated and the excess over a hundred surviving on the third day were killed. In stating the results of the experiments in numerical form it is difficult to make any very satisfactory correction for an excess of not-plague deaths. While it is evident that in experiment I, for example, the eleven rats which died on the two days following the day of inoculation, and to a less extent the four rats which died on the next day, had very little chance of dying of plague, and might reasonably be deducted from the number exposed to death; it is equally clear that, in experiment X, the eighteen rats which died from causes other than plague between the fourteenth and twenty-second days are in quite a different position, since the last plague death occurred on the twelfth day and all the rats which

subsequently died of other causes had had just as good a chance of dying of plague as any of the others. The chief plague mortality has usually been on the third and fourth days after inoculation, and we have endeavoured to obtain a more accurate statement of the mortality by deducting the rats dying of other causes on the day of inoculation and the two days following. There are, however, comparatively few experiments (I, V, VI, VII) in which the alteration makes a material difference in the percentage mortality. Working along these lines we have completed twenty-three experiments, the details of which follow.

*Experiment I.*

100 Madras rats			50 Madras mice			each received 1/100,000 gramme plague rat's spleen on 7 January, 1910.									
100 Bombay rats			50 Bombay mice												
76 Poona rats															
Date	Madras rats			Bombay rats			Poona rats			Madras mice			Bombay mice		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
1910															
Jan. 7	—	—	100	—	—	100	—	—	76	—	—	50	—	—	50
„ 8	5	—	95	—	—	100	—	—	76	7	—	43	—	—	50
„ 9	6	1	88	3	1	96	—	1	75	2	—	41	7	1	42
„ 10	4	47	37	5	20	71	3	4	68	—	13	28	1	19	22
„ 11	1	24	12	6	14	51	—	4	64	—	9	19	—	2	20
„ 12	—	8	4	—	2	49	—	3	61	1	6	12	—	2	18
„ 13	—	1	3	—	3	46	—	2	59	—	1	11	—	—	18
„ 14	—	2	1	—	1	45	—	3	56	—	—	11	—	—	18
„ 15	—	—	—	—	1	44	—	1	55	—	—	11	—	2	16
„ 16	1	—	0	—	—	44	1	1	53	—	—	11	—	1	15
„ 17	—	—	—	6	—	38	—	—	53	—	—	11	1	—	14
„ 18	—	—	—	1	1	36	—	1	52	—	—	11	—	—	14
„ 19	—	—	—	1	—	35	1	2	49	—	—	11	—	—	14
„ 20	—	—	—	1	—	34	—	—	49	—	—	11	—	—	14
„ 21	—	—	—	2	1	31	1	1	47	—	—	11	—	—	14
„ 22	—	—	—	—	—	31	1	—	46	—	—	11	—	—	14
„ 23	—	—	—	—	—	31	—	—	46	—	—	11	—	—	14
„ 24	—	—	—	2	—	29	—	—	46	—	—	11	—	—	14
Totals	17	83	0	27	44	29	7	23	46	10	29	11	9	27	14

*Percentages dead of plague :*

Madras rats	...	83 0/0 (93 0/0 *)
Bombay rats	...	44 0/0 (45 0/0 *)
Poona rats...	...	30 0/0
Madras mice	...	58 0/0 (71 0/0 *)
Bombay mice	...	54 0/0 (63 0/0 *)

\* Corrected for excessive not-plague mortality.

*Immunity of Wild Rats**Experiment II.*

50 Madras rats, 50 Bombay rats and 50 Poona rats put into godowns on 28 January, 1910. Into each godown were placed 5 rats inoculated with plague on 28 January, 5 more on 12 February and 3 more on 26 February. The deaths from plague were as follows:

Date, 1910	Bombay rats		Poona rats		Madras rats	
	Godown 1	Godown 2	Godown 3	Godown 4	Godown 5	Godown 6
28th Jan. — 3rd Feb.	0	0	0	0	0	0
4th Feb. — 10th „	0	0	0	0	4	1
11th „ — 17th „	0	1	0	0	3	3
18th „ — 24th „	0	1	0	1	3	11
25th „ — 3rd March	0	0	0	0	2	4
4th March — 14th „	0	1	0	0	3	2
Totals	0	3	0	1	15	21

*Percentages dead of plague:*

Bombay rats	...	6 %
Poona rats	...	2 %
Madras rats	...	72 %

*Average flea prevalence:*

Godown 1	...	4.2
„ 2	...	2.2
„ 3	...	4.4
„ 4	...	4.7
„ 5	...	4.3
„ 6	...	5.0

*Experiment III.*

12 Madras rats ... } were each inoculated with 1/20,000 gramme  
 48 wild Bombay rats ... } of plague rat's spleen on 5 May, 1910.  
 48 rats bred from Bombay rats in captivity }

The wild Bombay rats and the bred rats were matched in pairs as regards size and weight.

Date	Madras rats			Wild Bombay rats			Godown bred rats		
	Dead not plague	Dead of plague	Re-maining	Dead not plague	Dead of plague	Re-maining	Dead not plague	Dead of plague	Re-maining
May 5, 1910	—	—	12	—	—	48	—	—	48
„ 6 „	—	—	12	2	—	46	—	—	48
„ 7 „	—	1	11	2	—	44	—	—	48
„ 8 „	—	8	3	—	5	39	—	—	48
„ 9 „	—	3	0	—	8	31	—	4	44
„ 10 „	—	—	—	—	2	29	—	1	43
„ 11 „	—	—	—	—	1	28	—	3	40
„ 12 „	—	—	—	—	1	27	—	3	37
„ 13 „	—	—	—	—	—	27	—	2	35
„ 14 „	—	—	—	—	—	27	—	2	33
„ 15 „	—	—	—	—	—	27	—	1	32
„ 16 „	—	—	—	—	—	27	—	2	30
„ 17 „	—	—	—	—	—	27	—	—	30
Totals	0	12	0	4	17	27	0	18	30

*Percentages dead of plague:*

Madras rats	...	100 %
Wild Bombay rats	...	35 % (39 %)
Bred rats	...	37.5 %





*Experiment V.*

100 Madras rats ... }  
 100 Poona rats ... } were each inoculated with 1/20,000 gramme of plague  
 100 Bombay rats ... } rat's spleen on 8 July, 1910.  
 27 Madras bandicoots }

Date	Madras			Poona			Bombay			Bandicoots		
	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining
July 8, 1910	—	—	100	—	—	100	—	—	100	—	—	27
" 9 "	—	—	100	—	—	100	—	8	92	—	—	27
" 10 "	5	—	95	—	—	100	5	4	83	—	—	27
" 11 "	77	—	18	13	3	84	17	—	66	—	—	27
" 12 "	13	—	5	12	—	72	22	—	44	8	—	19
" 13 "	2	—	3	4	—	68	8	—	36	10	—	9
" 14 "	—	—	3	3	—	65	5	—	31	5	—	4
" 15 "	—	—	3	4	—	61	1	—	30	3	—	1
" 16 "	—	—	3	2	1	58	3	—	27	—	—	1
" 17 "	—	—	3	2	—	56	—	—	27	—	—	1
" 18 "	—	—	3	1	—	55	—	—	27	—	—	1
" 19 "	—	—	3	—	—	55	—	—	27	—	—	1
" 20 "	—	—	3	1	—	54	—	—	27	—	—	1
" 21 "	—	—	3	—	—	54	1	—	26	—	—	1
" 22 "	—	—	3	—	—	54	—	—	26	—	—	1
" 23 "	—	—	3	—	1	53	1	—	25	—	—	1
" 24 "	—	—	3	—	—	53	—	—	25	—	1	0
" 25 "	—	—	3	1	—	52	1	1	23	—	—	0
" 26 "	—	—	3	—	—	52	—	—	23	—	—	0
Totals	97	0	3	43	5	52	64	13	23	26	1	0

*Percentages dead of plague :*

Madras rats ... 97 %

Poona rats ... 43 %

Bombay rats... ... 64 % (73 %)

Madras bandicoots ... 96 %

*Experiment VI.*

100 Madras rats ... } were each inoculated with 1/50,000 gramme  
 100 young Poona rats... } of plague rat's spleen on 9 August,  
 100 young Bombay rats } 1910.

*Average weights:*

Madras ... 120 grammes  
 Young Poona ... 30 „  
 Young Bombay ... 30 „

Date	Madras rats			Young Poona rats			Young Bombay rats		
	Dead not plague	Dead of plague	Re- maining	Dead not plague	Dead of plague	Re- maining	Dead not plague	Dead of plague	Re- maining
Aug. 9, 1910	—	—	100	—	—	100	—	—	100
„ 10 „	—	—	100	11	—	89	—	—	100
„ 11 „	—	2	98	1	4	84	1	6	93
„ 12 „	—	4	94	—	5	79	—	9	84
„ 13 „	—	33	61	—	6	73	—	6	78
„ 14 „	—	29	32	—	11	62	—	3	75
„ 15 „	—	11	21	—	2	60	—	5	70
„ 16 „	—	6	15	—	5	55	—	5	65
„ 17 „	—	5	10	—	3	52	—	2	63
„ 18 „	—	3	7	—	1	51	—	6	57
„ 19 „	—	1	6	—	1	50	—	0	57
„ 20 „	—	2	4	—	0	50	—	3	54
„ 21 „	—	0	4	—	1	49	—	2	52
„ 22 „	—	1	3	—	0	49	—	0	52
„ 23 „	—	1	2	—	0	49	1	0	51
Totals	0	98	2	12	39	49	2	47	51

*Percentages dead of plague:*

Madras rats ... 98 %  
 Young Poona rats ... 39 % (44 %)  
 Young Bombay rats ... 47 %

Experiment VII.

100 Madras rats ... }  
100 young Poona rats } were each inoculated with 1/20,000 gramme of  
100 young Bombay rats } plague rat's spleen on 30 September, 1910.

Average weights:

Madras ... 94 grammes  
Young Poona ... 23 „  
Young Bombay ... 23 „

Date 1910	Young Poona rats			Young Bombay rats			Madras rats		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
September 30	—	—	100	—	—	100	—	—	100
October 1	28	—	72	4	—	96	—	—	100
„ 2	2	1	69	4	9	83	—	1	99
„ 3	—	1	68	—	5	78	—	43	56
„ 4	—	1	67	2	7	69	—	38	18
„ 5	2	5	60	3	16	50	—	9	9
„ 6	1	3	53	—	6	44	—	6	3
„ 7	2	3	48	—	4	40	—	—	3
„ 8	—	1	47	—	6	34	—	—	3
„ 9	—	—	47	—	2	32	—	—	3
„ 11	2	1	44	—	2	30	—	—	3
„ 12	—	—	44	—	—	30	—	—	3
„ 13	1	1	42	—	—	30	—	—	3
„ 14	—	—	42	—	—	30	—	—	3
„ 15	—	—	42	—	—	30	—	—	3
„ 16	—	—	42	—	—	30	—	—	3
„ 17	—	—	42	—	—	30	—	—	3
„ 18	—	—	42	—	—	30	—	—	3
„ 19	—	—	42	—	—	30	—	—	3
„ 20	—	—	42	—	—	30	—	—	3
Totals	38	20	42	13	57	30	0	97	3

Percentages dead of plague: .

Madras rats ... 97 %  
Young Poona rats ... 20 % (29 %)  
Young Bombay rats ... 57 % (62 %)

*Experiment VIII.*

100 Poona rats }  
100 Kirkee rats } were each inoculated with 1/5,000 gramme of plague rat's  
100 Bombay rats } spleen on 7 November, 1910

and

100 Madras rats received one-half the dose of the same emulsion.

Date	Poona			Kirkee			Madras			Bombay		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Nov. 7, 1910	—	—	100	—	—	100	—	—	100	—	—	100
„ 8 „	—	—	100	—	—	100	—	—	100	—	—	100
„ 9 „	—	2	98	—	1	99	—	12	88	—	4	96
„ 10 „	—	28	70	—	50	49	—	86	2	1	27	68
„ 11 „	—	25	45	—	22	27	—	1	1	—	19	49
„ 12 „	—	9	36	—	11	16	—	—	1	—	8	41
„ 13 „	—	7	29	—	7	9	—	—	1	—	9	32
„ 14 „	—	3	26	—	2	7	—	—	1	—	3	29
„ 15 „	—	1	25	—	1	6	—	—	1	—	—	29
„ 16 „	—	1	24	—	1	5	—	1	0	—	—	29
„ 17 „	—	0	24	—	—	5	—	—	0	—	—	29
„ 18 „	—	1	23	—	—	5	—	—	0	—	2	27
„ 19 „	—	—	23	—	—	5	—	—	0	—	—	27
„ 20 „	—	—	23	—	—	5	—	—	0	—	—	27
„ 21 „	—	—	23	—	—	5	—	—	0	—	1	26
Totals	0	77	23	0	95	5	0	100	0	1	73	26

*Percentages dead of plague :*

Madras rats	...	100 %
Kirkee rats	...	95 %
Poona rats	...	77 %
Bombay rats	...	73 %

*Experiment IX.*

100 Madras rats } were each inoculated with 1/5,000 gramme of  
 100 Poona rats } plague rat's spleen on 5 January, 1911.  
 100 Bombay rats }

Date	Madras rats			Poona rats			Bombay rats		
	Dead not plague	Dead of plague	Re- maining	Dead not plague	Dead of plague	Re- maining	Dead not plague	Dead of plague	Re- maining
Jan. 5, 1911	—	—	100	—	—	100	—	—	100
„ 6 „	—	—	100	—	—	100	—	—	100
„ 7 „	—	8	92	—	3	97	—	—	100
„ 8 „	—	79	13	1	13	83	2	38	60
„ 9 „	—	10	3	—	31	52	—	23	37
„ 10 „	—	3	0	—	14	38	—	5	32
„ 11 „	—	—	—	—	11	27	—	5	27
„ 12 „	—	—	—	—	4	23	—	3	24
„ 13 „	—	—	—	—	1	22	—	1	23
„ 14 „	—	—	—	—	—	22	—	2	21
„ 15 „	—	—	—	—	1	21	—	1	20
„ 16 „	—	—	—	—	—	21	—	—	20
„ 17 „	—	—	—	—	—	21	—	2	18
„ 18 „	—	—	—	—	—	21	—	—	18
„ 19 „	—	—	—	—	—	21	—	1	17
„ 20 „	—	—	—	—	—	21	—	1	16
Totals	0	100	0	1	78	21	2	82	16

*Percentages dead of plague:*

Madras rats	...	100 %
Poona rats	...	78 %
Bombay rats	...	82 %

Experiment X.

100 Madras rats ... }  
 100 Bombay rats ... } were each inoculated with 1/20,000 gramme of plague  
 100 Kirkee rats ... } rat's spleen on 16 January, 1911.  
 100 Poona rats ... }  
 23 Madras bandicoots }

Date 1911	Madras rats			Bombay rats			Poona rats			Kirkee rats			Madras bandicoots		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Jan. 16	—	—	100	—	—	100	—	—	100	—	—	100	—	—	23
„ 17	—	—	100	—	—	100	—	—	100	—	—	100	—	—	23
„ 18	—	3	97	—	—	100	—	—	100	—	—	100	—	—	23
„ 19	—	27	70	—	1	99	—	7	93	—	3	97	—	1	22
„ 20	—	38	32	—	5	94	—	3	90	—	9	88	—	1	21
„ 21	—	15	17	—	8	86	—	4	86	—	10	78	—	5	16
„ 22	—	6	11	—	5	81	—	5	81	—	10	68	—	9	7
„ 23	—	5	6	—	3	78	—	1	80	—	4	64	—	5	2
„ 24	—	0	6	—	3	75	—	2	78	—	2	62	—	1	1
„ 25	—	2	4	1	0	74	—	1	77	—	1	61	—	0	1
„ 26	—	0	4	—	—	74	—	1	76	—	—	61	—	—	1
„ 27	—	0	4	—	—	74	—	—	76	—	—	61	—	—	1
„ 28	—	1	3	—	1	73	—	—	76	—	—	61	—	—	1
„ 29	—	—	3	—	—	73	—	—	76	—	—	61	—	—	1
„ 30	—	—	3	3	—	70	—	1	75	—	—	61	—	—	1
„ 31	—	—	3	7	—	63	1	—	74	—	—	61	—	—	1
Feb. 1	—	—	3	—	—	63	—	—	74	—	1	60	—	—	1
„ 2	—	1	2	1	—	62	1	—	73	—	—	60	—	—	1
„ 3	—	—	2	3	—	59	4	—	69	1	—	59	—	—	1
„ 4	—	—	2	2	—	57	—	—	69	—	—	59	—	—	1
„ 5	—	—	2	—	—	57	—	—	69	1	—	58	—	—	1
„ 6	—	—	2	—	—	57	2	—	67	2	—	56	—	—	1
„ 7	—	—	2	2	—	55	2	—	65	—	—	56	—	—	1
Totals	0	98	2	19	26	55	10	25	65	4	40	56	0	22	1

Percentages dead of plague:

Madras rats	...	98 %
Bombay rats	...	26 %
Poona rats	...	25 %
Kirkee rats	...	40 %
Madras bandicoots	...	96 %

## Experiment XI.

100 young Poona rats } were each inoculated with 1/50,000 gramme  
 100 young Bombay rats } of plague rat's spleen on 14 February,  
 100 Madras rats } 1911.

Average weights :

Madras ... .. 110 grammes  
 Young Poona ... .. 40 „  
 Young Bombay ... .. 40 „

Date	Young Poona			Young Bombay			Madras		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Feb. 14, 1911	—	—	100	—	—	100	—	—	100
„ 15 „	—	—	100	—	—	100	—	—	100
„ 16 „	—	2	98	4	—	96	—	—	100
„ 17 „	—	6	92	—	10	86	—	71	29
„ 18 „	—	3	89	—	10	76	—	22	7
„ 19 „	—	5	84	—	13	63	—	5	2
„ 20 „	—	4	80	—	4	59	—	1	1
„ 21 „	—	5	75	—	6	53	—	1	0
„ 22 „	—	2	73	—	3	50	—	—	—
„ 23 „	—	2	71	—	2	48	—	—	—
„ 24 „	1	—	70	—	—	48	—	—	—
„ 25 „	—	—	70	—	—	48	—	—	—
„ 26 „	1	—	69	1	—	47	—	—	—
„ 27 „	—	—	69	—	—	47	—	—	—
„ 28 „	—	—	69	1	—	46	—	—	—
Totals	2	29	69	6	48	46	0	100	0

Percentages dead of plague :

Madras rats ... .. 100 %  
 Young Poona rats ... .. 29 %  
 Young Bombay rats ... .. 48 % (50 %)



Experiment XII.

100 Madras rats }  
 100 Dacca ,, } were each inoculated with 1/100,000 gramme of plague  
 100 Poona ,, } rat's spleen on 24 February, 1911.  
 100 Bombay ,, }

Date 1911	Madras rats			Dacca rats			Bombay rats			Poona rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Feb. 24	—	—	100	—	—	100	—	—	100	—	—	100
„ 25	—	—	100	—	—	100	—	—	100	—	—	100
„ 26	—	—	100	—	4	96	—	—	100	—	1	99
„ 27	3	85	12	—	45	51	—	16	84	—	14	85
„ 28	—	6	6	—	29	22	—	18	66	—	14	71
March 1	—	5	1	—	4	18	—	10	56	—	10	61
„ 2	—	1	0	—	6	12	2	1	53	3	5	53
„ 3	—	—	0	—	—	12	—	1	52	1	—	52
„ 4	—	—	0	—	—	12	—	1	51	—	1	51
„ 5	—	—	0	—	2	10	1	—	50	—	1	50
„ 6	—	—	0	—	—	10	—	1	49	2	—	48
„ 7	—	—	0	—	—	10	1	—	48	—	—	48
„ 8	—	—	0	—	2	8	—	—	48	—	—	48
„ 9	—	—	0	—	—	8	1	2	45	—	—	48
„ 10	—	—	0	—	—	8	—	1	44	—	—	48
„ 11	—	—	0	—	1	7	—	—	44	—	—	48
„ 12	—	—	0	—	—	7	2	—	42	—	—	48
„ 13	—	—	0	—	—	7	1	—	41	—	1	47
„ 14	—	—	0	—	—	7	—	—	41	—	—	47
„ 15	—	—	0	—	—	7	1	2	38	—	—	47
„ 16	—	—	0	—	—	7	—	—	38	1	1	45
Totals	3	97	0	0	93	7	9	53	38	7	48	45

Percentages dead of plague:

Madras rats	...	...	97 %
Dacca ,,	...	...	93 %
Bombay ,,	...	...	53 %
Poona ,,	...	...	48 %

*Experiment XIII.*

100 Madras rats  
 100 Dacca „  
 100 Poona „  
 100 Yeotgaon rats  
 100 Bombay „

} were each inoculated with 1/50,000 gramme  
 of plague rat's spleen on 10 March, 1911.

Date 1911	Madras rats			Dacca rats			Poona rats			Yeotgaon rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
March 10	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100
„ 11	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100
„ 12	—	4	96	—	—	100	—	—	100	—	1	99	—	—	100
„ 13	—	65	31	—	46	54	—	5	95	—	15	84	—	6	94
„ 14	—	25	6	—	36	18	—	5	90	—	21	63	—	9	85
„ 15	—	—	6	—	5	13	—	5	85	—	9	54	—	6	79
„ 16	—	—	6	—	3	10	—	8	77	—	9	45	—	6	73
„ 17	—	—	6	—	3	7	—	—	77	—	2	43	—	2	71
„ 18	—	2	4	—	—	7	3	2	72	—	1	42	—	1	70
„ 19	—	1	3	—	—	7	—	2	70	—	3	39	—	—	70
„ 20	—	—	3	—	1	6	2	1	67	—	2	37	—	4	66
„ 21	—	—	3	—	—	6	5	—	62	—	1	36	—	—	66
„ 22	—	—	3	—	3	3	2	2	58	—	3	33	—	1	65
„ 23	—	—	3	—	—	3	3	—	55	—	2	31	—	1	64
„ 24	—	—	3	—	—	3	—	1	54	1	—	30	1	—	63
„ 25	—	—	3	—	—	3	2	—	52	—	—	30	1	—	62
„ 27	—	1	2	—	—	3	2	—	50	—	1	29	2	—	60
„ 29	—	—	2	—	—	3	2	—	48	—	—	29	—	—	60
April 1	—	—	2	—	—	3	—	—	48	—	—	29	2	—	58
Totals	0	98	2	0	97	3	21	31	48	1	70	29	6	36	58

*Percentages of rats dead of plague :*

Madras rats	...	...	...	98 %
Dacca „	...	...	...	97 %
Poona „	...	...	...	31 %
Yeotgaon rats	...	...	...	70 %
Bombay „	...	...	...	36 %

Experiment XIV.

100 Madras rats ... } were each inoculated with 1/100,000 gramme of  
 50 Chit Baragaon rats ... } plague rat's spleen on 22 April, 1911.  
 100 Bombay rats ... }

Date	Madras rats			Chit Baragaon rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
April 23, 1911	—	—	100	—	—	50	—	—	100
„ 24 „	—	—	100	—	—	50	1	—	99
„ 25 „	—	19	81	—	—	50	—	—	99
„ 26 „	—	44	37	—	—	50	—	8	91
„ 27 „	—	19	18	—	1	49	—	4	87
„ 28 „	—	15	3	—	2	47	—	6	81
„ 29 „	—	1	2	—	2	45	—	1	80
„ 30 „	—	1	1	—	3	42	—	2	78
May 1 „	—	—	1	—	4	38	—	2	76
„ 2 „	—	—	1	—	3	35	—	—	76
„ 3 „	1	—	0	—	2	33	—	—	76
„ 4 „	—	—	—	—	—	33	—	—	76
„ 5 „	—	—	—	—	—	33	—	—	76
„ 6 „	—	—	—	1	1	31	2	—	74
„ 7 „	—	—	—	—	1	30	2	—	72
„ 8 „	—	—	—	—	—	30	1	—	71
„ 9 „	—	—	—	—	—	30	1	—	70
„ 10 „	—	—	—	—	—	30	1	—	69
„ 11 „	—	—	—	—	—	30	—	—	69
„ 12 „	—	—	—	—	—	30	—	—	69
„ 13 „	—	—	—	—	—	30	—	—	69
„ 14 „	—	—	—	—	—	30	—	—	69
„ 15 „	—	—	—	—	—	30	—	—	69
Totals	1	99	0	1	19	30	8	23	69

Percentages dead of plague :

Madras ... 99 %  
 Chit Baragaon ... 38 %  
 Bombay ... 23 %

## Immunity of Wild Rats

## Experiment XV.

100 adult Madras rats			100 Cawnpore rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats					
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
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100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
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50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
50 young Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 Lucknow rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats		
100 adult Madras rats			100 adult Bombay rats			100 young Bombay rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			100 young Poona rats			100 adult Poona rats			1		

Percentages dead of plague:

Adult Madras	...	95 %	Lucknow	...	37 %	Adult Bombay	...	19 %	Adult Poona	...	25 %
Young Madras	...	100 %	Cawnpore	...	15 %	Young Bombay	..	34 %	Young Poona	...	35 %

Experiment XVI.

Date, 1911	100 Madras rats			100 Bombay rats (adult)			50 " " (young)			50 Poona " "			50 Poona " "			100 Bombay rats (adult)			50 " " (young)			50 Poona " "		
	100 Madras rats			100 Madras rats			75 Cawnpore rats			75 Vaniyambadi rats			100 Bombay rats (adult)			50 " " (young)			50 Poona " "			100 Bombay rats (adult)		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
June 22	—	—	100	—	—	100	—	—	75	—	—	75	—	—	100	—	—	50	—	—	50	—	—	50
" 23	—	3	97	—	—	100	—	—	75	—	—	75	—	—	100	—	—	50	—	—	50	—	—	50
" 24	—	55	42	—	49	51	—	2	73	—	34	41	—	11	88	—	1	49	—	3	47	—	3	47
" 25	—	27	15	—	37	14	—	2	71	—	23	18	—	7	81	—	5	44	—	3	44	—	3	44
" 26	—	9	6	—	10	4	—	3	68	—	10	8	—	1	75	—	3	41	—	2	42	—	2	42
" 27	—	3	3	—	2	2	—	2	66	—	4	4	—	3	61	—	4	25	—	2	40	—	2	40
" 28	—	2	1	—	—	2	—	1	63	—	1	3	—	2	55	—	1	24	—	1	39	—	1	39
" 29	—	—	1	—	—	2	—	1	61	—	—	3	—	1	54	—	—	23	—	—	39	—	—	39
" 30	—	—	1	—	—	2	—	3	57	—	1	2	—	2	51	—	1	22	—	2	37	—	2	37
July 1	—	—	1	—	—	2	—	—	57	—	—	2	—	2	49	—	1	21	—	1	36	—	1	36
" 2	—	—	1	—	2	0	—	—	55	—	1	1	—	3	46	—	—	21	—	—	36	—	—	36
" 3	—	1	0	—	—	0	—	14	40	—	1	0	—	1	45	—	7	14	—	6	30	—	—	30
" 4	—	—	0	—	—	0	—	3	37	—	—	0	—	—	44	—	—	14	—	—	30	—	—	30
" 5	—	—	0	—	—	0	—	—	36	—	—	0	—	5	39	—	—	14	—	4	26	—	—	26
" 6	—	—	0	—	—	0	—	—	36	—	—	0	—	4	35	—	—	14	—	3	23	—	—	23
" 7	—	—	0	—	—	0	—	1	35	—	—	0	—	3	32	—	1	13	—	1	22	—	—	22
" 8	—	—	0	—	—	0	—	—	35	—	—	0	—	—	32	—	—	13	—	—	22	—	—	22
" 9	—	—	0	—	—	0	—	1	34	—	—	0	—	—	32	—	—	13	—	—	22	—	—	22
" 10	—	—	0	—	—	0	—	—	34	—	—	0	—	1	31	—	—	13	—	—	22	—	—	22
Totals	0	100	0	0	100	0	26	15	34	0	75	0	24	45	31	13	24	13	14	14	22	14	14	22

Percentages dead of plague :—Madras 100 %, Madras 100 %, Cawnpore 20 %, Vaniyambadi 100 %, adult Bombay 45 %, young Bombay 48 %, young Poona 28 %.



## Experiment XVIII.

100 Madras rats	} were each inoculated with 1/100,000 grammes of plague rat's spleen (=12,000 plague bacilli) on 1 September, 1911.
100 Raipur rats	
60 Cawnpore rats	
100 Calicut rats	
100 Palghat rats	
100 Poona rats	
100 Bombay rats	

Date	Madras rats			Rajpur rats			Cawnpore rats			Calicut rats			Palghat rats			Poona rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Sept. 1	—	—	100	—	—	100	—	—	—	—	—	100	—	—	100	—	—	—	—	—	100
" 2	—	—	100	—	—	100	—	—	—	—	—	100	—	—	100	—	—	—	—	—	100
" 3	—	1	99	—	—	100	—	2	98	—	1	99	—	—	100	—	—	—	—	—	100
" 4	—	13	86	—	25	75	—	1	78	—	14	85	—	—	100	—	—	—	—	—	100
" 5	—	44	42	—	52	23	—	2	48	—	36	49	—	5	95	—	—	—	—	3	97
" 6	—	15	27	—	13	10	—	4	33	—	14	35	—	6	89	—	—	—	—	5	92
" 7	—	12	15	—	8	2	—	4	29	—	12	23	—	3	86	—	—	—	—	5	87
" 8	—	4	11	—	2	0	—	1	20	—	8	15	—	2	84	—	—	—	—	2	85
" 9	—	2	9	—	2	—	—	1	16	—	2	13	—	1	83	—	—	—	—	2	85
" 10	—	—	9	—	—	—	1	—	15	—	—	13	—	—	83	—	—	—	1	—	83
" 11	—	—	9	—	—	—	—	—	15	—	1	12	—	—	83	—	—	—	1	—	82
" 12	—	1	8	—	—	—	1	—	13	—	—	11	—	—	83	—	—	—	—	—	82
" 13	—	1	7	—	—	—	1	—	12	—	1	11	—	—	80	—	—	—	—	—	81
" 14	—	—	7	—	—	—	1	—	12	—	—	11	—	—	78	—	—	—	—	—	80
" 15	—	—	7	—	—	—	1	—	11	—	1	11	—	—	78	—	—	—	—	—	79
" 16	—	—	7	—	—	—	—	—	11	—	—	11	—	—	78	—	—	—	—	—	78
" 17	—	—	7	—	—	—	—	—	11	—	—	11	—	—	78	—	—	—	—	—	77
" 18	—	—	7	—	—	—	—	—	11	—	—	11	—	—	78	—	—	—	—	—	77
" 19	—	—	7	—	—	—	—	—	11	—	—	11	—	—	78	—	—	—	—	—	76
" 20	—	1	6	—	—	—	—	—	10	—	—	11	—	—	78	—	—	—	—	—	75
" 21	—	1	5	—	—	—	—	—	9	—	1	10	—	—	76	—	—	—	—	—	75
Totals	0	95	5	0	100	0	4	10	91	9	1	89	10	6	18	76	9	16	75		

### Percentages dead of plague:

Madras	95 %	Cawnpore	17 % ;	Palghat	89 %	Bombay	16 %
Raipur	100 %	Calicut	91 %	Poona	18 %		

*Experiment XIX.*

100 Lucknow rats      100 Bombay rats      } each received 1/100,000 gramme plague  
 80 Cawnpore rats      90 Poona P.C. rats      } rat's spleen (=20,000 plague bacilli)  
 60 Banda rats      100 Poona N.P.C. rats      } on 28 October, 1911.

Date 1911	Lucknow			Cawnpore			Banda			Bombay			Poona P.C.			Poona N.P.C.		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
Oct. 28	—	—	100	—	—	80	—	—	60	—	—	100	—	—	90	—	—	100
„ 29	—	—	100	—	—	80	—	—	60	—	—	100	—	—	90	—	—	100
„ 30	—	1	99	—	—	80	—	1	59	—	3	97	—	1	89	—	1	99
„ 31	—	7	92	—	4	76	—	21	38	—	6	91	—	5	84	—	6	93
Nov. 1	—	14	78	—	7	69	—	23	15	—	12	79	—	7	77	—	5	88
„ 2	—	5	73	—	2	67	—	6	9	—	1	78	—	2	75	—	7	81
„ 3	—	2	71	—	3	64	—	4	5	—	4	74	—	6	69	—	3	78
„ 4	—	1	70	—	—	64	—	2	3	—	1	73	—	1	68	—	1	77
„ 5	—	1	69	—	—	64	—	1	2	—	1	72	—	4	64	—	1	76
„ 6	—	1	68	2	—	62	—	1	1	2	1	69	—	1	63	—	—	76
„ 7	—	—	68	—	—	62	—	—	1	—	1	68	—	2	61	—	—	76
„ 8	1	—	67	—	—	62	—	—	1	2	—	66	—	1	60	2	—	74
„ 9	2	—	65	—	—	62	—	—	1	—	—	66	—	—	60	—	—	74
„ 10	—	—	65	—	—	62	—	—	1	1	—	65	—	—	60	1	—	73
„ 11	—	—	65	—	—	62	—	—	1	—	—	65	—	—	60	1	—	72
„ 12	—	—	65	—	—	62	—	—	1	—	—	65	—	—	60	1	—	71
„ 13	—	—	65	—	—	62	—	—	1	—	—	65	—	—	60	—	—	71
„ 14	2	—	63	—	—	62	—	—	1	2	—	63	1	—	59	2	—	69
„ 15	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
„ 16	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
„ 17	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
„ 18	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
„ 19	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
Totals	5	32	63	2	16	62	0	59	1	7	30	63	1	30	59	7	24	69

*Percentages dead of plague :*

Lucknow	...	32 %	Bombay	...	30 %
Cawnpore	...	20 %	Poona P.C.	...	33 %
Banda	...	98 %	Poona N.P.C.	...	24 %

} 28 %



Experiment XX.

100 Madras rats      100 Bombay rats  
 50 Lucknow rats    100 Nagpur rats  
 100 Madras rats    50 Surat rats  
 100 Poona rats

each received 1/100,000 grammes plague rat's spleen (=18,000 plague bacilli) on 25 November, 1911.

Date 1911	Madras			Lucknow			Madras			Poona			Bombay			Nagpur			Surat		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
Nov. 25	—	—	100	—	—	50	—	—	100	—	—	100	—	—	100	—	—	100	—	—	50
" 26	—	—	100	—	—	50	—	—	100	—	—	100	—	—	100	—	—	100	—	—	50
" 27	—	—	95	—	—	50	—	—	100	—	—	98	—	—	95	—	—	94	—	—	49
" 28	—	—	79	—	—	50	—	—	76	—	—	95	—	—	90	—	—	81	—	—	47
" 29	—	—	36	—	—	45	—	—	24	—	—	93	—	—	77	—	—	57	—	—	43
" 30	—	—	22	—	—	43	—	—	11	—	—	90	—	—	72	—	—	31	—	—	39
Dec. 1	—	—	14	—	—	40	—	—	8	—	—	88	—	—	64	—	—	19	—	—	36
" 2	—	—	8	—	—	39	—	—	1	—	—	86	—	—	62	—	—	11	—	—	34
" 3	—	—	6	—	—	34	—	—	6	—	—	86	—	—	59	—	—	6	—	—	34
" 4	—	—	2	—	—	33	—	—	6	—	—	86	—	—	57	—	—	4	—	—	33
" 5	—	—	5	—	—	32	—	—	5	—	—	82	—	—	55	—	—	3	—	—	30
" 6	—	—	—	—	—	31	—	—	5	—	—	81	—	—	52	—	—	2	—	—	29
" 7	—	—	—	—	—	29	—	—	5	—	—	80	—	—	52	—	—	2	—	—	28
" 8	—	—	—	—	—	28	—	—	5	—	—	76	—	—	50	—	—	2	—	—	27
" 9	—	—	—	—	—	26	—	—	5	—	—	76	—	—	48	—	—	2	—	—	27
" 10	—	—	—	—	—	26	—	—	5	—	—	73	—	—	47	—	—	2	—	—	26
" 11	—	—	—	—	—	25	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
" 12	—	—	—	—	—	25	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
" 13	—	—	—	—	—	24	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
" 14	—	—	—	—	—	24	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
" 15	—	—	—	—	—	24	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
Totals	0	96	4	10	16	24	0	95	5	7	20	73	12	42	46	1	98	1	3	21	26

\* Chronic plague.

Percentages dead of plague:

Madras	Lucknow	Madras	Poona	Bombay	Nagpur	Surat
96 %	32 %	95 %	20 %	42 %	98 %	42 %

## Experiment XXI.

100 Madras rats ...  
 100 Bombay rats ...  
 80 Poona rats ...  
 75 Muzaffarnagar rats ...  
 80 Delhi Shahdara rats ...

each received 1/100,000 gramme plague rat's spleen  
 (=16,000 plague bacilli) on 31 January, 1912.

Date 1912	Madras			Muzaffarnagar			Delhi Shahdara			Poona			Bombay		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
Jan. 31	—	—	100	—	—	75	—	—	80	—	—	80	—	—	100
Feb. 1	—	—	100	—	—	75	—	—	80	—	—	80	—	—	100
„ 2	—	—	100	—	1	74	—	—	80	—	—	80	—	—	100
„ 3	—	12	88	—	—	74	—	—	80	—	3	77	—	4	96
„ 4	—	30	58	—	4	70	—	3	77	—	2	75	—	—	96
„ 5	—	20	38	—	4	66	—	5	72	—	2	73	—	4	92
„ 6	—	13	25	—	2	64	—	3	69	—	3	70	—	2	90
„ 7	—	10	15	—	4	60	—	7	62	—	—	70	—	5	85
„ 8	—	2	13	—	2	58	—	1	61	—	2	68	—	5	80
„ 9	—	1	12	—	1	57	—	1	60	—	1	67	—	—	80
„ 10	—	2	10	—	—	57	—	—	60	—	—	67	—	1	79
„ 11	—	2	8	1	—	56	1	—	59	2	—	65	1	—	78
„ 12	—	2	6	—	—	56	—	1	58	—	—	65	—	1	77
„ 13	—	2	4	—	1	55	—	1	57	—	1	64	—	2	75
„ 14	—	1	3	2	—	53	1	—	56	2	—	62	—	—	75
„ 15	—	—	3	1	1	51	—	1	55	1	—	61	1	—	74
„ 16	—	—	3	—	—	51	—	—	55	—	—	61	—	—	74
„ 17	—	—	3	—	—	51	—	—	55	—	—	61	—	—	74
„ 18	—	—	3	1	—	50	—	—	55	—	—	61	1	—	73
„ 19	—	1	2	—	—	50	—	—	55	—	—	61	—	—	73
„ 20	—	—	2	—	—	50	—	—	55	—	—	61	—	—	73
„ 21	—	—	2	—	—	50	—	—	55	—	—	61	—	—	73
Totals	0	98	2	5	20	50	2	23	55	5	14	61	3	24	73

Percentages dead of plague :

Madras	Muzaffarnagar	Delhi Shahdara	Poona	Bombay
98 %	27 %	29 %	17 %	24 %



Experiment XXIII.

90 Madras rats  
97 Belgaum rats  
98 Banda rats  
100 Bombay rats

} each received 1/100,000 gramme of a plague rat's spleen  
(= 20,000 plague bacilli) on 27 May, 1912.

Date 1912	Madras			Belgaum			Banda			Bombay		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
May 27	—	—	90	—	—	97	—	—	98	—	—	100
„ 28	—	—	90	—	—	97	—	—	98	—	—	100
„ 29	—	—	90	—	—	97	—	—	98	—	—	100
„ 30	—	8	82	—	2	95	—	10	88	—	2	98
„ 31	—	33	49	—	1	94	—	32	56	—	5	93
June 1	—	20	29	—	3	91	—	19	37	—	4	89
„ 2	—	11	18	—	1	90	—	16	21	—	2	87
„ 3	—	6	12	—	2	88	—	4	17	—	4	83
„ 4	—	2	10	—	1	87	—	4	13	—	1	82
„ 5	—	2	8	—	—	87	—	2	11	—	1	81
„ 6	—	1	7	—	—	87	—	2	9	—	—	81
„ 7	—	1	6	1	—	86	—	1	8	2	—	79
„ 8	—	—	6	—	—	86	—	—	8	1	—	78
„ 9	—	2	4	—	—	86	—	—	8	—	—	78
„ 10	—	—	4	—	—	86	—	—	8	1	—	77
„ 11	—	1	3	1	—	85	—	1	7	—	1	76
„ 12	—	—	3	—	—	85	—	—	7	—	—	76
„ 13	—	—	3	—	—	85	—	—	7	—	—	76
„ 14	—	—	3	—	—	85	—	—	7	—	1	75
„ 15	—	—	3	1	—	84	—	—	7	—	—	75
„ 16	—	—	3	—	—	84	—	—	7	—	—	75
„ 17	—	—	3	—	—	84	—	—	7	—	—	75
Totals	0	87	3	3	10	84	0	91	7	4	21	75

Percentages dead of plague:

Madras	Belgaum	Banda	Bombay
97 %	10 %	93 %	21 %

As regards adult wild rats, the results are summarised in table I and in table II is given shortly the plague history of the places from which the rats were derived. Their geographical relations are shown in the appended map.

Table I shows clearly that the susceptibility to fatal plague infection varies a great deal in rats from different places, and by comparison with table II it appears that the resistance of the rats varies in a general way with the local prevalence of plague in recent years. The places investigated fall into three main groups.

(1) *Places which have suffered severely and almost continuously from plague for about ten years or more.* Bombay rats have been compared with the standard susceptible Madras rats in 18 inoculation experiments with an average mortality of 38 % against 97 % in the Madras rats. Rats from Poona have in the same way been tested against Madras and Bombay rats on 14 occasions by inoculation: the total result is that of 1390 Madras rats 1347 died (97 %), of 1387 Bombay rats 533 (38 %) and of 1355 rats from Poona only 446 (33 %). Similarly the resistance of rats from the two other large plague-infected towns of Cawnpore and Lucknow was found to be of a high grade, the summed figures of comparative experiments being

Madras, 485 out of 500	= 97 %.
Bombay, 173 out of 598	= 29 %.
Poona, 138 out of 587	= 24 %.
Cawnpore, 75 out of 414	= 18 %.
Lucknow, 117 out of 349	= 34 %.

Other towns in this group were investigated less frequently. Belgaum, where plague has for many years been exceptionally severe, yielded rats only 10 % of which died<sup>1</sup>. Surat (Bombay), Bellary (Madras), Bandsdeh (United Provinces) and Chit Baragaon (United Provinces) gave figures (37 to 42 % mortality) rather higher than those for Bombay (29 %) in parallel experiments, as also did Kirkee. Two other places in the United Provinces were examined—Muzaffarnagar and Delhi Shahdara—and in both places the rats were found about as little susceptible as those of Bombay. Nagpur alone of the places with a long history of severe plague gave susceptible rats, 97 and 98 % dying in two experiments. Pending further enquiries this result must be regarded as exceptional. The rats, however, were not collected

<sup>1</sup> In an experiment already published (vol. x. p. 458) Bombay, Poona and Belgaum rats were exposed to infected fleas: there died 18, 27 and 9 per cent. respectively.



Map showing the places from which rats were obtained for immunity experiments.

TABLE I. Showing the percentage mortality among wild adult *Mus rattus* from various places.

Number	Date	Dose	Madras	Bombay	Poona	Kirkee	Chit Barragon	Bansdeh	Cawnpore	Lucknow	Bellary	Bhagalpur	Nagpur	Belgaum	Surat	Muzaffarnagar	Delhi Shahdara	Coimbatore	Calicut	Palghat	Podanur	Vaniyambadi	Raipur	Banda	Madura	Dacca	Yeotgaon	Number
I	Jan. 7, 1910	1/100,000 grm.	93	45	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	I	
II	Jan. 28	" flea infection	72	6	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	II	
III	May 5	" 1/20,000 grm.	100	39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	III	
IV	June 2	" 1/20,000 "	95	26	10	—	—	—	—	—	—	57	—	—	—	—	—	—	—	—	—	—	—	—	—	—	IV	
V	July 8	" 1/20,000 "	97	73	43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	V	
VI	Aug. 9	" 1/50,000 "	98	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	VI	
VII	Sept. 30	" 1/20,000 "	97	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	VII	
VIII	Nov. 7	" 1/5,000 "	100	73	77	95	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	VIII	
IX	Jan. 5, 1911	" 1/5,000 "	100	82	78	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	IX	
X	Jan. 16	" 1/20,000 "	98	26	25	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X	
XI	Feb. 14	" 1/50,000 "	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XI	
XII	Feb. 24	" 1/100,000 "	97	53	48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	93	—	XII	
XIII	Mar. 10	" 1/50,000 "	98	36	31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	97	70	XIII	
XIV	Apr. 22	" 1/100,000 "	99	23	—	—	38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XIV	
XV	May 18	" 1/100,000 "	95	19	25	—	—	—	15	37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XV	
XVI	June 21	" 1/100,000 "	100	45	—	—	—	—	20	—	—	—	—	—	—	—	—	—	—	—	100	—	100	—	—	—	XVI	
XVII	Aug. 2	" 1/100,000 grm.= 26,000 bacilli	99	21	21	—	—	37	19	32	—	—	97	—	—	—	—	—	—	—	—	—	—	—	—	—	XVII	
XVIII	Sept. 1	" 1/100,000 grm.= 12,000 bacilli	95	16	18	—	—	—	17	—	—	—	—	—	—	—	—	—	91	89	—	—	100	—	—	—	XVIII	
XIX	Oct. 28	" 1/100,000 grm.= 20,000 bacilli	—	30	28	—	—	—	20	32	—	—	—	—	—	—	—	—	—	—	—	—	—	98	—	—	XIX	
XX	Nov. 25	" 1/100,000 grm.= 18,000 bacilli	96	42	20	—	—	—	—	32	—	—	98	—	42	—	—	—	—	—	—	—	—	—	95	—	XX	
XXI	Jan. 31, 1912	" 1/100,000 grm.= 16,000 bacilli	98	24	17	—	—	—	—	—	—	—	—	—	—	27	29	—	—	—	—	—	—	—	—	—	XXI	
XXII	Apr. 27	" 1/100,000 grm.= 14,000 bacilli	97	27	13	—	—	—	—	—	40	—	—	—	—	—	—	60	—	—	92	—	—	—	83	—	XXII	
XXIII	May 27	" 1/100,000 grm.= 20,000 bacilli	97	21	—	—	—	—	—	—	—	—	—	12	—	—	—	—	—	—	—	—	93	—	—	—	XXIII	

TABLE II. *Plague deaths per 10,000.*

	Population 1901	1897	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	Years infected	Average rate since com- mencement
Bombay <sup>1</sup>	776,006	136	213	202	181	208	214	269	173	191	145	88	68	68	44	55	16	149
Poona <sup>2</sup>	153,320	221	27	668	280	101	182	441	304	80	467	72	85	9	nil	41	13	213
Kirkee <sup>3</sup>	10,797	—	610	4	164	8	68	80	216	27	1	262	3	266	nil	nil	12	244
Chit Baragaon	9,505	—	—	—	—	—	425	1	197	95	394	173	18	127	173	369	10	197
Bansdeh	10,024	—	—	—	—	—	261	7	245	109	2	337	204	176	212	67	10	171
Cawnpore	183,712	—	—	—	—	—	396	282	187	103	149	62	32	47	42	106	10	141
Lucknow	264,049	—	—	—	—	—	—	160	136	206	15	234	nil	nil	nil	47	6	89
Bellary	58,247	—	—	—	—	138	241	123	167	19	1	135	1	$\frac{1}{2}$	8	66	11	82
Bhagalpur <sup>4</sup>	75,760	—	—	—	—	$\frac{1}{2}$	$\frac{1}{2}$	34	200	74	144	12	89	$\frac{1}{2}$	nil	nil	9	50
Nagpur <sup>5</sup>	127,734	—	—	—	—	—	—	755	376	7	427	12	76	571	22	150	9	274
Belgaum <sup>6</sup>	26,237	109	1120	814	600	392	541	418	324	132	29	33	165	145	156	93	15	338
Surat	119,306	—	160	4	6	$\frac{3}{4}$	24	244	22	341	5	43	3	nil	$\frac{1}{2}$	$\frac{1}{2}$	13	61
Muzaffarnagar <sup>7</sup>	—	—	—	—	—	—	—	12	60	189	33	567	9	2	46	340	9	140
Delhi Shahdara <sup>7</sup>	—	—	—	—	—	—	2	24	19	76	3	151	7	$\frac{2}{5}$	22	254	10	56
Coimbatore	53,080	—	—	—	—	—	—	106	141	1	$\frac{1}{2}$	nil	nil	204	10	142	7	67
Calicut	76,981	—	—	—	—	—	—	$\frac{1}{2}$	nil	nil	nil	nil	26	10	3	7	5	5
Palghat	44,177	—	—	—	—	—	—	—	$\frac{1}{2}$	$\frac{1}{2}$	nil	nil	nil	22	7	nil	4	4
Podanur	6,568	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaniyambadi	12,908	—	—	—	—	594	745	1058	7	nil	nil	nil	nil	nil	nil	nil	4	219
Madras <sup>8</sup>	509,346	—	—	—	—	—	—	—	—	$\frac{1}{2}$	nil	nil	nil	nil	nil	nil	1	0.08
Raipur <sup>9</sup>	35,335	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	5	21
Banda	22,565	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Madura	105,984	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dacca <sup>10</sup>	90,679	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Yeotgaon	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

No plague

<sup>1</sup> Vol. vii. p. 726, vol. viii. p. 267, this volume, p. 221.<sup>4</sup> Vol. xi. supplement, p. 184.<sup>7</sup> Details for these villages not available. The mortalities may be taken to be those of the Mozuffarnagar (see vol. x. p. 349, vol. xi. supplement, p. 106) and Meerut districts respectively, which are given above.<sup>8</sup> This volume, p. 209.<sup>2</sup> Vol. viii. p. 268, vol. x. p. 483.<sup>5</sup> Vol. viii. p. 269.<sup>3</sup> Vol. x. p. 484.<sup>6</sup> Vol. viii. p. 270, vol. x. p. 446.<sup>10</sup> Vol. xi. supplement, p. 173.



under the immediate supervision of the Commission and it may be that they came from a part of the town which has been relatively free from plague.

(2) *Places which have suffered relatively slightly from plague.* Chief among these is Madras City which has been free from plague except for one small outbreak in the outskirts of the city in 1905. The susceptibility of the rats from here is sufficiently evident. Raipur (Central Provinces) also falls in this group and has very susceptible rats. All the other five places are in the Madras presidency: Vaniyambadi suffered from two very severe epidemics some time since but has been free from plague for eight years. Calicut, Palghat and Podanur have had several small outbreaks scattered over a number of years, and the rats from all four towns proved highly susceptible. They were rather more resistant from Coimbatore, where plague prevailed rather extensively during the three years preceding the time when the rats were obtained for experiment.

(3) *Places which have been free from plague.* The four places under this heading all yielded susceptible rats. It is of some importance to note that they are widely scattered about India. The rats from Banda (United Provinces), Madura (Madras) and Dacca (Assam) were as little immune as those from Madras City. A figure was obtained indicating a rather greater resistance, though much less than in Bombay, Poona and other places in group 1, for Yeotgaon, a village in the immediate neighbourhood of Poona in which on careful enquiry we could obtain no evidence that plague had ever been present.

In comparing the figures in table I with one another it should be noted that the degrees of immunity indicated are relative to the dose, and that no very exact comparison can be made between the results of one experiment and those of another even if the dose is nominally the same since it cannot be assumed that the infectivity of the bacilli was in all cases constant. The experiments (XVII to XXIII) in which the bacilli were enumerated in a constant quantity of emulsions, made from spleens of acute plague rats showing approximately an equal number of bacilli on microscopical examination, show that  $\frac{1}{100}$  mgm. contained from 12,000 to 26,000 bacilli. This is perhaps under the circumstances a very satisfactory degree of uniformity, but at the same time the range of variation shows that a dose of  $\frac{1}{20}$  mgm. cannot be regarded as being necessarily greater than one of  $\frac{1}{50}$  mgm. With wide differences in dosage, however, the difference in effect is plain enough:  $\frac{1}{5}$  mgm. (experiments VIII and XI) killed 78 % of Bombay rats and 77 of

those from Poona, as against 42 and 26 % with  $\frac{1}{20}$  mgm., 31 and 24 % with  $\frac{1}{100}$  mgm., and 6 and 2 % with flea infection<sup>1</sup>. It should also be understood that the dose which suitably brings out the difference between batches of rats of widely varying resistance is not necessarily an appropriate quantity for the examination of animals more nearly alike. Thus a dose of  $\frac{1}{100}$  mgm. killed on the average 97 % of Madras rats, and this mortality could not be much increased under any circumstances: it rises to the limit with  $\frac{1}{5}$  mgm. and falls to 72 % in the flea-infection experiment. All the rats from Vaniyambadi and Raipur were killed by the same dose of  $\frac{1}{100}$  mgm. and more than 90 % from Nagpur, Calicut, Podanur, Banda, Madura and Dacca, but it does not follow that the resistance was in all cases approximately the same. Further experiments with smaller doses may show considerable variation in the rats from these different localities. In the case of Kirkee  $\frac{1}{5}$  mgm. killed 95 % but a second trial with  $\frac{1}{20}$  mgm. showed that the rats from this place possessed a pretty high degree of resistance. Similarly larger doses might show that the animals from Poona, Cawnpore and Belgaum really differ more widely than the present series indicates.

Taking the series of experiments as a whole it seems clear that the *resistance of rats to plague is associated with the past prevalence of plague*, and is not due to racial and local variations in susceptibility. There is of course no doubt that among a series of batches of rats all conforming to the type of *Mus rattus* it is possible to discover special and characteristic features in the specimens from certain places, not as a rule wholly confined to any one locality but being predominant in one place more than in another<sup>2</sup>. Thus the fur of Madras City rats is exceptionally rufous, and a very large proportion of Palghat rats had white fur on the under surface of the body while this feature was extremely rare in Poona. Rats from the colder northern places had thicker fur than southern specimens, and Belgaum rats were always on the whole larger than those caught in Bombay or Poona. But no external differences could be detected in the rats from Poona, Kirkee and Yeotgaon, which are situated within a few miles from one another. Poona has suffered severely from plague, Kirkee less severely and Yeotgaon not at all, and we found that the immunity of the rats from each place was proportionate to the degree of plague prevalence. Similarly

<sup>1</sup> The difficulty of conveying infection by a single flea emphasises the importance of dosage in this means of infection (see vol. vii. p. 411).

<sup>2</sup> See "The Races of Indian Rats," by R. E. Lloyd, in *Records of the Indian Museum*, vol. iii. part 1, and especially p. 84.

with respect to Cawnpore, Lucknow and Banda in the United Provinces. It was not possible to distinguish the rats of Cawnpore or Lucknow from those of Banda by external appearances, but on testing them by inoculation Banda rats proved to be as susceptible as Madras rats, and Cawnpore and Lucknow rats both highly immune, the former markedly more than the latter. The plague histories of the three places have been widely different; the disease has been nearly twice as severe in Cawnpore as in Lucknow, and epidemics have recurred yearly in Cawnpore while in some recent years Lucknow has escaped. Banda has been entirely free from the disease. Any local or racial variations in resistance to plague which may be associated with morphological differences such as we have indicated seem therefore to be obscured in the present series of experiments, and, as will be seen from the map, there is no evidence of any geographical distribution of immunity. Highly susceptible rats were found in such widely separated parts of India as Madras, Dacca, Madura and Banda, and highly immune animals in Bombay, Belgaum and Cawnpore. It would also be an extraordinary coincidence if plague had visited just those places where the rats were naturally most immune to the disease and spared those with the most susceptible rat populations.

It will not have escaped notice that the rats of Vaniyambadi proved to be very susceptible in 1911 and that the place had suffered from exceptionally severe plague in 1901—1903. Evidently therefore any immunity resulting from these epidemics had disappeared in seven or eight years. On the other hand we have evidence that resistance may persist during a period free from plague. In Poona the last evidence of indigenous plague was obtained in March 1909 and though the place was kept under careful observation with continuous rat examination, it remained free from plague until September 1911, a period of some 30 months. An epidemic of moderate severity then began, 630 deaths being recorded up to the end of 1911 and a further 457 by April 1912, when the outbreak came to an end. The immunity experiments cover the period from January 1910 to April 1912 and there is nothing in the results which suggests that the resistance of the rats underwent any alteration during that period. We have no definite information as to the length of life of *Mus rattus* in nature, but if the data obtained in the breeding experiments (vol. IX. supplement, p. 205) indicate with only approximate accuracy the natural rate of growth the average rat used for experimental purposes could not be more than about 6 months old. Hence in January 1910 the great majority at any rate of the rats

tested may be presumed to have been born since the disappearance of plague from the rat population of Poona<sup>1</sup>, and in the later experiments of 1911 there can be no doubt that this became more absolutely true in the sense that many of the animals would not only have never had a chance of experiencing epidemic plague themselves, but would be the grandchildren or great grandchildren rather than the sons and daughters of any rats which ever had. Hence we reach the conclusion that *the resistance found is not due merely to the previous reception by the experimental rats of sublethal and immunising doses of plague bacilli during the prevalence of epidemic plague but to the generation of a relatively immune progeny by the survivors of epidemics.*

This conclusion is supported by two further sets of experiments. In the first place we tested the resistance of quite young rats, from Madras, Bombay and Poona, which can have been only a few weeks old. The results show that the immunity of these baby rats is of the same

TABLE III. *Showing the percentage mortality among young wild rats.*

Experi- ment	Date	Dose	Madras		Bombay		Poona	
			Adult	Young	Adult	Young	Adult	Young
VI.	Aug. 9, '10	1/50 mgm.	98	—	—	47	—	39 (44*)
VII.	Sept. 30, '10	1/20 „	97	—	—	57 (62*)	—	20 (29*)
XI.	Feb. 14, '11	1/50 „	100	—	—	48 (50*)	—	29
XV.	May 18, '11	1/100 „	95	100	19	34	25	35
XVI.	June 21, '11	1/100 „	100	—	45	48	—	28

\* Corrected for non-plague mortality during first three days.

order as that of the average adult rat from the same place, whether that place is and has been free from plague (Madras), is and has been heavily infected with plague (Bombay) or is free from plague with a history of severe infection in the past (Poona). Absolutely the young rats seem to be definitely rather more susceptible than the corresponding adults receiving an equal dose: this is confirmed by other experiments detailed below (p. 265).

In the second place young rats were obtained by breeding from wild rats in flea-proof godowns at Parel (vol. XI. supplement, p. 193), and as far as the results go they show that the young reproduce the resistance of their parents with remarkable fidelity.

We do not think that there is any reason to suppose that this inherited resistance is due to rats becoming immunised during an epidemic and transmitting this immunity to their offspring. The facts

<sup>1</sup> For some further evidence on this point, see below, p. 279 and note.

TABLE IV. *Showing the percentage mortality among young rats bred in captivity.*

Experiment	Date	Dose	Madras		Bombay	
			Wild	Bred	Wild	Bred
III.	May 5, '10	1/20 mgm.	100	—	35	37
XVII.	Aug. 2, '11	1/100 „	99	—	21	20
XXII.	April 27, '12	1/100 „	97	100	27	22

are more easily explained by supposing that the susceptible members of a rat population are destroyed during an epidemic and that subsequent generations continue, for a time at any rate, to exhibit the resistance of the survivors. The average immunity of the rats after an epidemic will therefore in a general way be proportional to its severity, dispersion and duration, a mild epidemic leaving many rats of moderate resistance alive and a severe outbreak destroying all but those of the highest immunity. The natural process appears to be the same as that which we have already investigated in connection with the experimental godown epizootics. Thus in one series (vol. x. p. 332) 74 of 143 wild Bombay rats survived flea-borne infection in the godowns and none of them died when they were subsequently inoculated with the same dose of spleen emulsion which killed 68 of 137 (50 %) control rats. Similar results were obtained in subsequent experiments (this volume, p. 298), and the details are given above (p. 253, experiment XXII) of a test in which none of 30 survivors of experimental epidemics died with a dose which killed 97 % of Madras, 27 % of Bombay and 13 % of Poona rats. From other survivors 25 young rats were bred in captivity (p. 248, experiment XVII): of these one only died as against 99 %, 21 % and 21 % of Madras, Bombay and Poona rats respectively. If the same process went on in nature, it is easy to see how the 2 or 3 %, or with larger doses presumably the one per thousand or ten thousand, of the rat population of a place such as Madras which lived through a severe epidemic would quickly give rise to a highly resistant race<sup>1</sup>.

#### *Experiments on other animals.*

Incidentally a few comparative experiments were also made with Madras bandicoots (experiments V and X) and with mice from Madras and Bombay (experiment I). The former show a susceptibility equal, with the doses used, to that of Madras rats (see also vol. VII. p. 760, vol. x. p. 459). Mice on the other hand proved relatively immune, whether they came from a plague-infected or a plague-free place.

<sup>1</sup> For the possible rate of reproduction of rats see vol. xi. supplement, p. 198.

TABLE V. *Showing the percentage mortality from plague of bandicoots and mice compared with rats.*

Experiment	Dose	Madras rats	Bombay rats	Poona rats	Madras mice	Bombay mice	Madras bandicoots
V.	1/20,000	97	64 (73 *)	43	—	—	96
X.	1/20,000	98	26	25	—	—	96
I.	1/100,000	83 (93 *)	44	30	58 (71 *)	54 (63 *)	—

\* Corrected for early mortality from causes other than plague: see p. 232, above.

## SUMMARY.

1. *Rats from different places show varying degrees of immunity to plague.*
2. *This immunity is relative to the infecting dose.*
3. *Immunity is greatest in places which have suffered most severely from plague, less marked in those places which have suffered to a moderate extent and least in those places in which epidemic plague has not occurred.*
4. *This immunity is not always acquired from an attack of plague, and is transmitted by the parents to their offspring which have not been exposed to infection.*

## APPENDIX.

*On the influence of size and sex on the percentage mortality.*

Experiments such as those detailed above have involved the use of rats varying somewhat in size; quite small animals were, however, never used except in the particular experiments designed to test the resistance of young rats. It is important therefore to know whether a moderate variation in size or any sexual differences in the various batches of rats would be likely to lead to any considerable variation in the results of inoculation, the same dose being used whatever the size of the animal. The pretty regular uniformity in the results for Madras and Bombay rats with which many experiments were made is evidence that these factors have not introduced any very material error. We give here, however, the analysis of another series of observations made in Poona in 1909 with the idea of testing for seasonal changes in resistance (see vol. x. p. 523). All the animals received as far as possible the same dose of spleen emulsion. Table VI gives the results for 2000 consecutive rats (77 of which died from causes other than plague) in groups of 500 classified by weight, and table VII deals with the same animals grouped also by sex.

TABLE VI.

Weight grammes	Jan. 22 to Feb. 28	March 2 to April 13	April 13 to May 18	May 19 to June 25	Total inocu- lated	Total died of plague	Per cent. died of plague
11—30	15/45 = 33 %	25/61 = 41 %	43/84 = 51 %	39/80 = 49 %	270	122	45.2
31—50	23/67 = 34	38/128 = 30	15/54 = 28	38/82 = 46	331	114	34.4
51—70	11/58 = 19	12/48 = 25	28/78 = 36	28/83 = 34	267	79	29.6
71—90	32/65 = 49	15/53 = 28	22/70 = 31	17/67 = 25	255	86	33.7
91—110	43/112 = 38	19/88 = 22	25/92 = 27	23/65 = 35	357	110	30.8
111—130	23/57 = 40	11/49 = 22	10/52 = 19	18/59 = 31	217	62	28.6
131—150	15/41 = 37	6/38 = 16	11/42 = 26	13/41 = 32	162	45	27.8
151—170	5/15 = 33	7/13 = 54	1/9 = 11	3/11 = 27	48	16	33.3
171—210	2/4 = 50	0/4 = 0	0/4 = 0	2/4 = 50	16	4	25.0
Totals	169/464 = 36 %	133/482 = 28 %	155/485 = 32 %	181/492 = 37 %	1923	638	33.2 %

It appears from this that young rats are definitely more susceptible than older individuals<sup>1</sup>, the dose being the same for all weights. The difference is however in no way proportional to the weight and is small relatively to the differences dealt with above in rats from different

TABLE VII.

Weight grammes	Males			Females		
	Inoculated	Died	Per cent.	Inoculated	Died	Per cent.
71— 90	109	38	35	146	48	33
91—110	141	50	35	216	60	28
111—130	89	29	32	128	33	26
131—150	88	28	32	74	17	23
151—170	30	12	40	18	4	22
171—210	13	3	23	3	1	33
Totals	470	160	= 34.0 %	585	163	= 27.9 %

places. Among grown-up rats there is no definite relation between weight and percentage mortality. As regards sex, table VII shows that females are apparently somewhat less resistant than males, but the difference is not sufficient to enable one to definitely exclude the errors of chance selection.

<sup>1</sup> At the time these experiments were made, some of the older animals had probably had experience of a plague epidemic.

### LII. CHRONIC OR RESOLVING PLAGUE.

IN previous numbers of these reports (vol. VI. p. 580, vol. VII. pp. 457, 719, vol. X. p. 335) some remarks have been made and observations recorded on the condition called chronic plague, or, as we prefer to name it, resolving plague. In order to obtain a more complete knowledge of this condition, we decided to make a careful examination daily of some two hundred rats (100 *decumanus* and 100 *rattus*) caught alive in Bombay city. We proposed to carry on this examination for a whole year. At the same time we continued to gain experience of this condition in other places in India, for example at Poona, and we were able to compare the frequency and character of the lesions we found in rats in these plague-infected centres with similar lesions found in rats in the plague-free city of Madras.

#### I. *Resolving plague in Bombay.*

The first part of this paper deals with our observations in Bombay city. In our early experience of plague we failed to observe or at least to note the lesions we now recognise to be often produced by the plague bacillus and which we have called resolving plague lesions. This was not to be wondered at for several reasons, among the most important of which was the fact that these resolving lesions have very little resemblance to the lesions found in the acute stages of the disease; they were therefore passed over as due to some unknown cause. Moreover, in our early examination of rats in Bombay, the work was so organised that, while the animals were cut up by one individual, a detailed bacteriological examination was made some hours later by another. In the meantime the surface of the abscesses and other lesions became contaminated with organisms which in cultures inhibited or obscured the growth of the plague bacillus<sup>1</sup>. We also relied almost altogether on the cutaneous method of infection in guinea-pigs when we subjected any

<sup>1</sup> Reference should be made here to the single case of chronic plague which was noted in our early observations in Bombay: the case was detected by isolating the plague bacillus from a mixed culture by the use of a special medium (vol. VII. p. 468).



suspected material to animal tests. We now know that this method of infection is not often successful in demonstrating the presence of the comparatively small number of living plague bacilli found in these resolving plague lesions. But as our experience enlarged, especially when we came to deal with an increased number of rats which had been trapped alive and not found dead (as the majority were which we examined in Bombay in the early part of our observations), the relation of the resolving plague lesions to the acute lesions gradually unfolded itself. With the fuller knowledge gained by this extended experience we decided to re-examine the Bombay rats for evidence of resolving plague.

In our last paper (vol. x. p. 340) we endeavoured to lay down certain rules to enable us to distinguish and classify acute plague lesions from resolving lesions. We recognised that the rules were arbitrary and that a series of plague-infected rats might be selected which showed almost imperceptible gradations from the characteristic acute lesions to the very different resolving lesions. We hoped, however, for convenience of classification and comparison, by aid of these rules to divide plague-infected rats into three groups, viz. those with (*a*) acute lesions, (*b*) resolving lesions, (*c*) resolved lesions. Experience during the observations now about to be detailed, however, showed that the distinction we endeavoured to make between acute plague and the early stages of resolving plague was too fine to be practical and another group was therefore added to the series, viz. rats with appearances intermediate between the typical acute lesions and the well-marked and localised lesions of resolving plague. The term "subacute plague" was used to designate this group of rats. Our difficulties in classification, however, did not end here for, since no arbitrary line of distinction could be drawn between the rats of the different groups and as the observations extended over periods of more than a year, a uniform classification became almost impossible. A careful perusal of the notes, which were compiled from day to day, leaves little room for doubt that the grouping of the rats at the end of the observations was not just exactly the same as at their commencement, and this was particularly the case with the rats classed in the groups acute, subacute and resolving plague. For convenience of record, however, some classification was necessary in order to deal conveniently with the large amount of material we had collected. Little harm will result from errors in classification if this explanation serves to impress on the reader a fact which was patent to the workers, that the lesions we have attempted to classify under

separate heads formed in reality a continuous series of pathological changes produced, for the most part by the plague bacillus, from acute, through healing, to completely healed lesions.

Table I shows the number of rats examined each month in Bombay from September 1909 to August 1910, *decumanus* and *rattus* separately. The figures show the number found with (a) acute plague, (b) subacute plague, (c) resolving plague, (d) resolved, recovered or post-plague lesions. Table II gives a more detailed classification of the resolving and resolved lesions. It is hardly necessary to explain that many rats showed more than one of the lesions represented in the classification adopted in tables I and II, but each rat was recorded under one head only. A rat for example which showed perisplenitis, a scar in the spleen and adhesions between the spleen and the abdominal wall was classed under the head adhesions. Again, a rat which showed an abscess in the spleen with scars, perisplenitis and adhesions was recorded under the heading abscess of the spleen, that is under the heading which, in our opinion, represented the earliest stages in the process of resolution.

Before analysing further the material we have collected and before describing a few typical examples of the lesions recorded under each head, it remains only to remark that the observations in Bombay extended from the 16th August, 1909, to the 19th September, 1910. The rats had always been trapped alive and to all appearance were healthy before they were killed. As far as possible 100 *decumanus* and 100 *rattus* were examined daily except on Sundays and on certain other holidays. In the early part of the observations it was not possible to collect 100 rats of each species, but later the full number was obtained daily. In the case of rats suffering from acute or subacute plague no note was made of the species found infected, but in the case of rats suffering from resolving or resolved lesions complete notes were kept. It is interesting to observe that *M. decumanus* showed rather more than twice as many individuals with lesions under these heads as *M. rattus*. We have shown elsewhere (vol. VII. p. 750) that *M. decumanus* suffers rather more than twice as severely from acute plague as does *M. rattus*. This is probably explained by the fact that the average number of fleas found on *M. decumanus* in Bombay is rather more than twice as large as on *M. rattus* (see also vol. VIII. p. 297). Since all the rats we examined in the present series were trapped alive and since *decumanus* suffered rather more than twice as severely from resolving and resolved plague lesions than *rattus* did, the facts are in favour of the difference in

TABLE I.

Month	Number of rats examined							Resolving plague				Resolved plague			
	<i>decumanus</i>	<i>rattus</i>	Total	No. of acute plague rats	Per cent. of acute plague rats	No. of subacute plague rats	Per cent. of subacute plague rats	No. of <i>decumanus</i>	Per cent. of <i>decumanus</i>	No. of <i>rattus</i>	Per cent. of <i>rattus</i>	No. of <i>decumanus</i>	Per cent. of <i>decumanus</i>	No. of <i>rattus</i>	Per cent. of <i>rattus</i>
Sept. ...	2057	2089	4146	1	·02	0	0	12	·58	8	·38	21	1·02	11	·52
Oct. ...	2454	2500	4954	0	0	0	0	17	·69	3	·12	20	·81	12	·48
Nov. ...	2088	2200	4288	0	0	1	·02	21	1·06	6	·27	24	1·15	8	·36
Dec. ...	2000	2000	4000	0	0	0	0	15	·75	6	·30	31	1·15	12	·6
Jan. ...	2300	2300	4600	0	0	8	·17	14	·6	2	·08	50	2·19	11	·47
Feb. ...	2400	2400	4800	0	0	35	·73	16	·66	4	·16	36	1·5	33	1·37
March ...	2100	2100	4200	1	·02	35	·83	10	·48	5	·24	26	1·23	24	1·14
April ...	2500	2500	5000	4	·08	48	·96	16	·64	3	·12	32	1·28	21	·84
May ...	2500	2500	5000	10	·2	41	·82	4	·16	1	·04	34	1·36	11	·44
June ...	2400	2400	4800	2	·04	25	·52	10	·42	1	·04	48	2·00	25	1·04
July ...	2500	2500	5000	12	·24	5	·1	12	·48	2	·08	42	1·69	23	·92
Aug. ...	2400	2400	4800	6	·12	2	·04	12	·5	2	·08	43	1·8	26	1·08
Totals ...	27699	27889	55588	36	·06	200	·36	159	·57	43	·15	407	1·47	217	·77

TABLE II.

Month	No. of rats examined			Resolving plague						Post-plague lesions							
				Chronic buboes		Abscesses of spleen		Necrotic areas in spleen		Adhesions		Scars		Bisected spleen		Perisplenitis	
	<i>decumanus</i>	<i>rattus</i>	Total	<i>decumanus</i>	<i>rattus</i>	<i>decumanus</i>	<i>rattus</i>	<i>decumanus</i>	<i>rattus</i>	<i>decumanus</i>	<i>rattus</i>	<i>decumanus</i>	<i>rattus</i>	<i>decumanus</i>	<i>rattus</i>	<i>decumanus</i>	<i>rattus</i>
Sept. ...	2057	2089	4146	8	3	3	3	1	2	10	11	3	0	3	0	5	0
Oct. ...	2454	2500	4954	10	0	0	1	5	2	12	10	1	0	4	1	3	1
Nov. ...	2088	2200	4288	12	5	6	0	5	1	12	6	3	0	5	0	4	2
Dec. ...	2000	2000	4000	9	3	1	0	5	3	13	8	10	0	8	1	0	3
Jan. ...	2300	2300	4600	6	1	4	0	4	1	21	8	12	1	8	0	9	2
Feb. ...	2400	2400	4800	6	1	5	2	5	1	17	24	10	6	5	1	4	2
March ...	2100	2100	4200	2	1	0	0	8	4	18	20	1	3	4	1	3	0
April ...	2500	2500	5000	6	1	5	2	5	0	22	18	4	0	5	1	1	2
May ...	2500	2500	5000	0	0	0	0	4	1	18	11	5	0	8	0	3	0
June ...	2400	2400	4800	2	0	4	1	4	0	30	23	6	0	8	1	4	1
July ...	2500	2500	5000	2	0	4	2	6	0	24	19	6	3	6	1	6	0
Aug. ...	2400	2400	4800	4	0	6	0	2	2	26	19	9	5	6	1	2	1
Totals ...	27699	27889	55588	67	15	38	11	54	17	223	177	70	18	70	8	44	14

the number of fleas being the explanation of the observed difference in severity of the disease in the two species rather than the alternative explanation, which was at the same time advanced, that, owing to its habits, *decumanus* was more likely to be found dead by the collecting staff than *rattus*.

The lesions described under the heading "resolving plague" are, we believe, of comparatively recent development, that is to say they have not been present in the rat for many months. They are generally the result of an attack of plague from which the rat is recovering. These lesions always afford some morbid material which is capable of being submitted to bacteriological examination. It is not, however, always possible to prove that these lesions are due to the plague bacillus either because it is sometimes difficult to obtain an uncontaminated culture from them, or because the lesions have become sterile or secondarily infected by some other organism than the plague bacillus. It must be clearly understood, however, that while we believe that the majority of these lesions were originally produced by the plague bacillus, even although it is not always possible to demonstrate the plague bacillus in them, we feel sure that similar lesions to those produced by the plague bacillus are brought about by some other cause or causes. Evidence to this effect will be forthcoming when we describe our findings in the rats caught in Madras city.

#### A. *Resolving plague.*

We classify resolving lesions into three groups: (1) chronic buboes, (2) necrotic areas in the spleen, (3) chronic abscesses in the spleen and more rarely in the liver.

##### (a) *Chronic buboes.*

Discussing first chronic buboes, a detailed analysis has been made of 78 rats presenting these lesions. In two of this number the condition was associated with necrotic areas in the spleen and in two cases with resolved lesions in the spleen. Of the buboes 68 were cervical, nine were inguinal and one was axillary<sup>1</sup>. The buboes were generally single, and varied in size from a small pea to a haricot bean, the smaller ones being the commoner. Generally they were firm and hard, with thick gristly walls, and when incised were found to contain

<sup>1</sup> I.e. the distribution was the same as in acute plague rats (vol. VII. pp. 329, 386, 388, 826, 829, 910, vol. X. p. 469).

a small bead of pus. The pus was, in some cases, of a pale yellow colour and creamy in consistence, but in other cases the material contained in the buboes was dry and inspissated. *B. pestis*, when found microscopically, was present in those glands containing fluid pus, not in those containing inspissated material. The buboes were usually firmly fixed to the surrounding tissues, but there was no local congestion, oedema or haemorrhage surrounding them.

Of the 78 rats examined, the contents of the buboes of seven when injected subcutaneously into guinea-pigs produced plague in these animals. Five of these buboes were cervical, one inguinal and one axillary.

In two cases the guinea-pig died in three days.

In three cases                   "                   "                   six days.

In one case                   "                   "                   seven days.

In one case                   "                   "                   nineteen days.

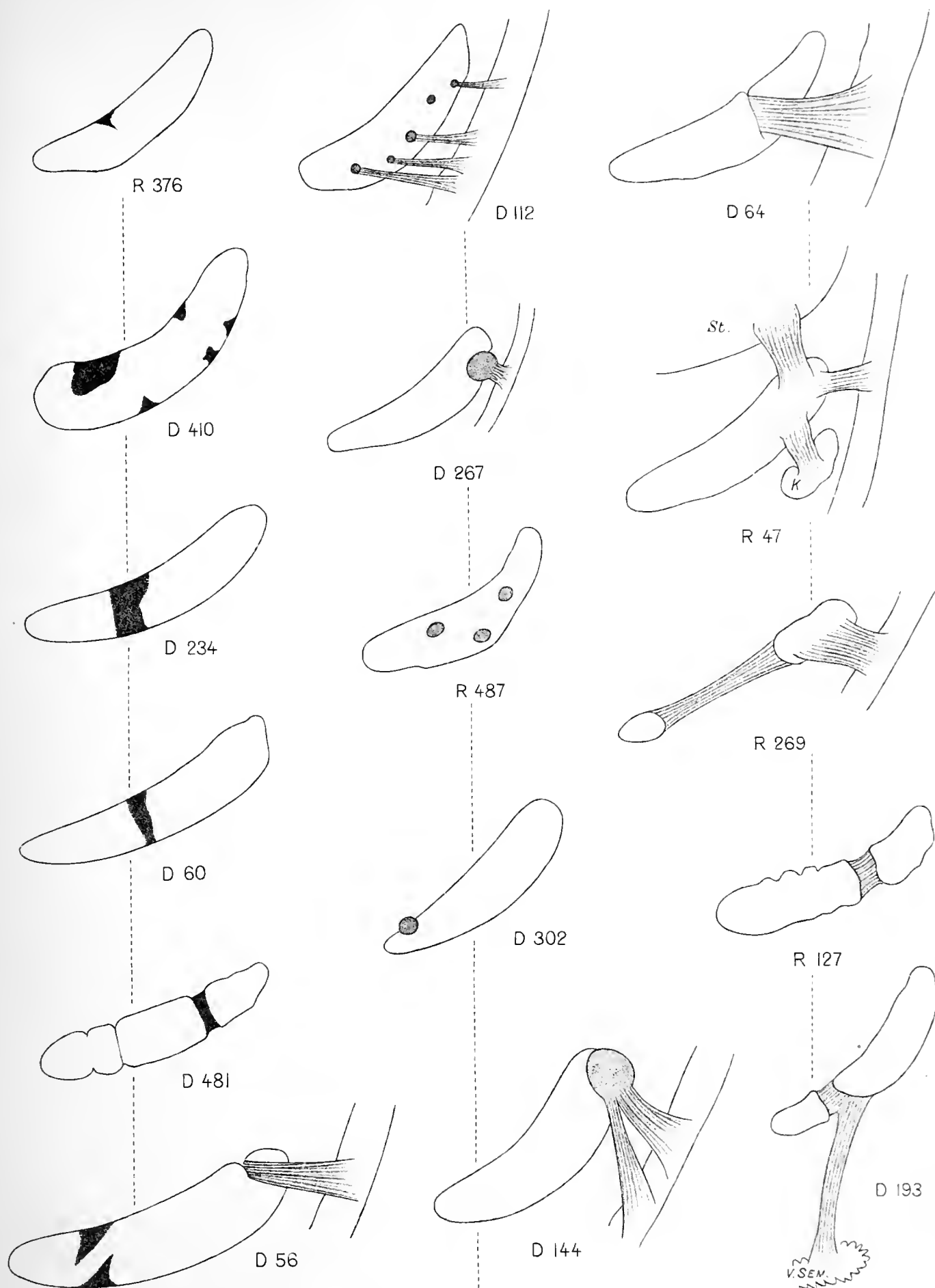
In seven cases a pure culture of *B. pestis* was obtained while material from the buboes failed to kill guinea-pigs. In 27 cases cultures from the buboes proved to be sterile. In 14 cases plague-like bacilli were seen microscopically in smears made from the buboes, and in five of these cases the cultures were nevertheless sterile and in nine others a growth was obtained which was not the plague bacillus. In the remaining 23 cases no evidence of plague was obtained by microscopical, cultural or animal tests, and cultures gave a growth other than plague.

It will thus be seen that about 9% of the buboes contained a sufficient number of plague bacilli sufficiently virulent to kill guinea-pigs when the pus from the buboes was injected subcutaneously into them. Further 9% of the buboes contained plague bacilli which were isolated by culture while the contents of the buboes failed to kill guinea-pigs. In 18% of the cases organisms which resembled the plague bacilli were seen microscopically in smears, but that they were really plague bacilli was not proved by culture or animal experiment. In 64% of the cases no definite evidence was obtained to show that the lesions were produced by the plague bacillus.

(b) *Necrotic areas in the spleen.*

An analysis has been made of 74 rats which showed necrotic areas in the spleen; two of these cases, as has been remarked above, were associated with chronic buboes. The necrotic conditions in the spleen were of four types. (1) Small nodules, about the size of a millet seed

or a lentil, situated as a rule just beneath the capsule of the spleen. They are well defined and of a firm consistence and are simply rather larger and more clearly defined than the necrotic spots found in the spleen and liver in acute plague (vol. VII. p. 332). The necrotic spots, as they enlarge and pus forms within them, give rise to the abscesses in the spleen described below. (2) Wedge-shaped necrotic areas were met with; their shape and appearance suggested that they had been produced by infarction. The base of the wedge was usually at the upper or lower margin of the spleen and the apex extended for a varying distance across the organ, often reaching half-way through its substance. These areas were of a pale yellow colour tinged with pink or of a more distinctly pink colour. In some cases the outline of the organ was preserved uninterruptedly over the necrotic area, but in other cases, especially those in which organisation and cicatrisation had commenced, an indentation of the border was produced which, as healing progressed, remained as a scar on the surface of the spleen. Rats showing scars of this kind have been classed among those with resolved or post-plague lesions, *i.e.* when all the necrosed tissues had been absorbed. (3) Band-shaped necrotic areas extended right across the spleen, generally completely through its substance. They varied in breadth from a narrow strip to a broad band, and in colour and appearance resembled the wedge-shaped necrotic areas described above. In some cases these bands bulged out from the surface of the spleen, in other cases they did not alter the contour of the organ. In those cases in which the healing process had advanced, and in which cicatrisation had taken place, the spleen became constricted at the site of the lesion. Complete organisation of these lesions led to the formation of a transverse scar, a condition we have classified as bisected spleen among the post-plague lesions. In some cases these band-shaped necrotic areas were multiple, and we have found transverse scars in the spleen associated with other bands which had not completely cicatrised, and in some of these cases we were able to demonstrate the presence of plague bacilli in the necrosed tissue which remained. (4) Large irregular necrotic areas were infrequent, but they sometimes involved as much as two-thirds to three-quarters of the entire area of the spleen, only small portions of healthy tissue being left. When such lesions had healed portions of the spleen were sometimes left widely separated from one another but connected together by strands of fibrous tissue. Of the 74 rats with necrotic areas in the spleen, 11 were shown to harbour living plague bacilli by injecting an emulsion of the necrosed tissue subcutaneously into guinea-pigs.



*Necrotic areas in spleen  
Inoculation positive with  
376, 234, 481 and 56.*

*Abscesses in spleen  
Inoculation positive with  
112, 487, 302 and 144.*

*Recovered plague with  
adhesions to abdominal  
wall etc.  
All inoculations negative.*





One guinea-pig died of plague on the second day after injection.

One	"	"	third	"	"
Three guinea-pigs	"	"	fourth	"	"
One guinea-pig	"	"	sixth	"	"
Two guinea-pigs	"	"	ninth	"	"
One guinea-pig	"	"	eleventh	"	"

Two more were chloroformed on the 19th and 26th days after injection and were found to be recovering from an attack of plague. In two cases, in which the material which had been injected into guinea-pigs had failed to produce plague in them, the plague bacillus was isolated from the lesions in pure culture. Twenty-four cases proved to be sterile. Three showed plague-like organisms microscopically, but other tests designed to detect the presence of the bacillus failed. In 34 cases growths other than plague were obtained, and other tests for plague were negative. It thus appears that about 15 % of the necrotic areas examined contained the *B. pestis* as demonstrated by the guinea-pig test. In 3 % of the cases *B. pestis* was isolated in cultures although the animal test failed. In 4 % bacilli resembling plague bacilli were seen microscopically, although this finding was not confirmed by other tests. 32 % of the cases proved to be sterile. In 78 % no direct evidence of plague was obtained.

(c) *Abscesses in the spleen or liver.*

Passing on to consider abscesses in the spleen we found that these varied in size from a small pea to an almond but a few large abscesses containing as much as three drachms of pus were met with. The abscesses were generally single but occasionally a few to many were found in the spleen of one rat. The walls of the abscesses were often thick and fibrous, and definitely marked off from the surrounding splenic tissue. In 25 instances the abscesses were fixed to adjoining structures by bands of adhesion. These adhesions most often extended between the abscess and the abdominal wall, and next in frequency between the abscess and the mesentery. In a few cases the abscesses were adherent to the stomach, intestines, liver, or kidney. The abscesses usually contained thick yellowish white pus. In some cases the pus was inspissated and dry. Bacteriological examination of 36 of the abscesses gave the following result. The contents of the abscess injected subcutaneously into guinea-pigs produced plague in these animals in 15 instances. In two cases, while the material injected into guinea-pigs failed to kill the animals, a pure culture of *B. pestis* was obtained from the

lesions. In five cases plague-like bacilli were seen in smears, but no other proof of their existence in the abscess was obtained. In four cases the contents of the abscesses were sterile. In fourteen (39 %) rats no evidence of the presence of the plague bacillus was obtained, and the cultures in these cases yielded other organisms than the plague bacillus. The results of the examination of these splenic abscesses shows that 42 % of them contained sufficiently numerous or sufficiently virulent plague bacilli to cause the development of the disease in guinea-pigs.

The series of cases shows (a) that some cases in which proof of the presence of plague bacilli was obtained resembled very closely others in which no evidence of the presence of these bacilli was forthcoming. (b) They show the relation which exists between this type of lesion and those described under the term resolved plague. In the latter cases the healing process has progressed a stage further; the plague bacilli have been destroyed, pus and necrotic tissue have been absorbed, adhesions, scars and perisplenitis remain the sole evidence of previous infection. There is no longer any material from which living plague bacilli can be isolated.

#### B. *Resolved or post-plague lesions.*

The conditions which we have to describe under this head are almost wholly connected with the spleen. They may be classified as follows :

- (1) Adhesions of the spleen to surrounding structures.
- (2) Scars in the spleen.
- (3) Bisected or trisected spleen.
- (4) Fibrous thickening of the capsule of the spleen, "perisplenitis."

(1) *Adhesions* were the most common of the four conditions. They were found between the spleen and surrounding structures in the following order of frequency :

(1)	To the abdominal wall	...	...	301 cases.
(2)	To the omentum	...	...	110 „
(3)	To the left kidney	...	...	35 „
(4)	To the stomach	...	...	23 „
(5)	To the uterus and ovary	...	...	17 „
(6)	To the vesiculae seminales	...	...	7 „
(7)	To the intestines	...	...	7 „
(8)	To the liver	...	...	4 „
(9)	To the bladder	...	...	1 case.

Adhesions of the abdominal wall and the omentum were often present together and an adhesion to one of the abdominal organs was often associated with adhesion to the abdominal wall.

The adhesions in some cases consisted of a firm matting of the spleen to the adjacent structures so that the organ was rendered quite immovable. In other cases fibrous bands stretched between the spleen and adjoining structures, only partially fixing the spleen. These bands were of various degrees of thickness and strength. Multiple adhesions were frequently present, they almost always arose from some scar on the surface of the spleen or from patches of perisplenitis.

(2) *Scars* were present, singly or combined with other lesions, especially with adhesions. The commonest situation for scars was near one or other border of the spleen, and there the margin of the spleen was indented. These scars and indentations were frequently multiple and in some cases caused considerable distortion of the spleen. Some scars extended transversely across the spleen, generally completely through the whole thickness of the organ. Occasionally small depressed scars were met with on the surface of the spleen where apparently necrotic spots had once existed.

(3) *Bisected spleens*, as the term indicates, were divided into two portions, the two parts being separated by a definite interval filled in by scar tissue. The separation of the two parts varied in degree, being as much as one inch in one case. Trisection was noted in a few cases. Adhesions frequently extended from the bisecting scar to the abdominal wall or to one of the abdominal organs.

(4) *Perisplenitis* generally existed as a well-defined area of pearly-grey colour. These areas varied much in size, in some cases they covered the greater portion of the surface of the spleen, but in other cases the areas were more numerous but smaller. Perisplenitis was often associated with scars in the spleen.

*An analysis of some cases in which resolving lesions were associated with resolved lesions.*

Resolving plague lesions as has been stated above were frequently associated with resolved lesions. The following are details of some of the combinations found:

Splenic abscess with adhesion to the abdominal wall	...	10 cases.
„ „ adhesion to the omentum	... 4 „	
„ „ adhesions to the kidney	... 2 „	

Splenic abscess with	adhesion to the stomach	...	...	1 case.
"	" adhesion to the intestines	...	...	2 cases.
"	" adhesions to the abdominal wall, stomach			
	and liver	...	...	2 "
"	" adhesion to the abdominal wall and			
	stomach	...	...	2 "
Necrotic spots	" adhesion to the abdominal wall...	...	...	5 "
"	" adhesion to the abdominal wall, bisected			
	spleen and scar	...	...	1 case.
Necrotic spot	" adhesion to the abdominal wall and			
	uterus	...	...	1 "
Necrotic spots	" adhesions to the omentum	...	...	5 cases.
Necrotic spot	" scar	...	...	1 case.
"	" perisplenitis	...	...	1 "

## II. *Observations in Poona.*

We may pass on now to record our experience of resolving and resolved plague in Poona city. Our observations in this place extended from June 1908 to March 1912. During this period 141,287 rats were examined. All these rats were trapped alive in the city of Poona and were all *Mus rattus*. The observations here are particularly interesting, since they covered two epidemics of the disease, one at the commencement and one at the close of the observations with a practically plague-free interval of nearly two and a half years between the two epidemics. During this interval very careful inquiries showed that the disease was neither epidemic nor epizootic in the city save only on two occasions when some evidence was collected to prove that limited epizootics were present, started probably by the importation of infection from outside the city. The one epizootic occurred in a part of the city called Nana's Peth early in November 1909, and the other in Shanwar Peth in March 1911. All the evidence goes to show that neither of these epizootics extended beyond the rats living in one or two houses in the immediate vicinity of one another.

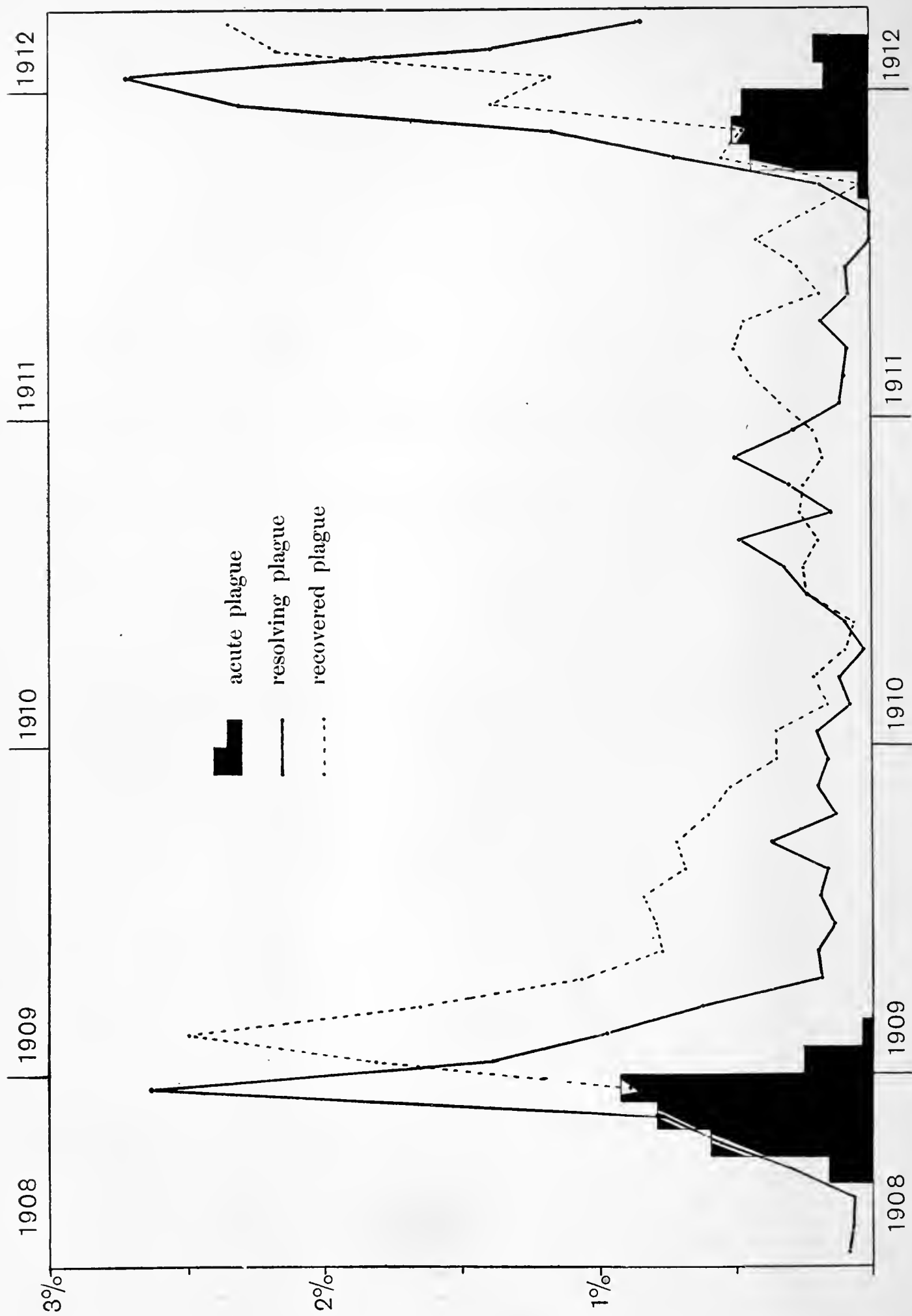
What has been said above in regard to difficulties in classification of the lesions found in Bombay rats applies with equal force to the investigations in Poona. Here the early observations from June to December 1908 are unreliable as to the number of rats found with resolving and resolved plague lesions. These early observations were largely instrumental, as has been explained, in unfolding to us the relation of these

TABLE III.

Table showing the number and percentage of acute plague, resolving plague (visceral and peripheral lesion) and recovered plague (adhesions only) met with in Poona *Mus rattus* caught alive in regular trapping since June 1908.

Month	Total live <i>rattus</i> examined	Acute plague alive		Resolving plague		Recovered plague (only adhesions)	
		Number	Monthly percentage	Number	Monthly percentage	Number	Monthly percentage
June 1908	3977	—	—	3	0·08	—	—
July „	5351	—	—	4	0·07	—	—
August „	5946	—	—	4	0·07	—	—
September „	6115	10	0·16	18	0·29	—	—
October „	4913	29	0·59	27	0·55	—	—
November „	3814	30	0·78	29	0·76	3	0·08
*December „	2923	27	0·92	77	2·63	26	0·88
January 1909	2376	6	0·25	33	1·39	43	1·81
February „	2284	1	0·04	22	0·97	57	2·50
March „	2906	—	—	18	0·62	48	1·65
April „	2644	—	—	5	0·19	28	1·06
May „	2479	—	—	5	0·20	19	0·77
June „	2830	—	—	4	0·14	22	0·78
July „	3727	—	—	7	0·19	31	0·83
August „	3554	—	—	6	0·17	24	0·68
September „	3520	—	—	13	0·37	25	0·71
October „	3508	—	—	5	0·14	21	0·60
November „	4059	—	—	8	0·20	21	0·52
December „	4306	—	—	7	0·16	15	0·35
January 1910	3462	—	—	7	0·20	12	0·35
February „	3659	—	—	3	0·08	6	0·16
March „	3273	—	—	4	0·12	7	0·21
April „	3120	—	—	1	0·03	3	0·10
May „	2875	—	—	3	0·10	2	0·07
June „	2916	—	—	7	0·24	7	0·24
July „	3140	—	—	10	0·32	8	0·25
August „	3534	—	—	17	0·48	7	0·20
September „	2712	—	—	4	0·15	7	0·26
October „	3628	—	—	11	0·30	9	0·25
November „	4408	—	—	22	0·50	8	0·18
December „	4298	—	—	12	0·28	9	0·21
January 1911	3380	—	—	4	0·12	11	0·33
February „	2929	—	—	3	0·10	13	0·44
March „	3290	—	—	3	0·09	17	0·50
April „	2775	—	—	5	0·18	13	0·47
May „	2622	—	—	2	0·08	5	0·19
June „	2211	—	—	2	0·09	6	0·27
July „	2703	—	—	0	—	11	0·41
August „	2631	—	—	0	—	6	0·23
September „	2765	1	0·04	5	0·18	1	0·04
October „	1840	8	0·43	13	0·71	10	0·54
November „	2401	12	0·50	28	1·16	11	0·46
December „	1953	9	0·46	45	2·30	27	1·38
January 1912	1802	3	0·17	49	2·72	21	1·17
February „	1527	3	0·20	21	1·38	33	2·16
March „	1201	—	—	10	0·83	28	2·33
Total	148287	139	0·09	586	0·40	681	0·46

\* Owing to difficulties in classification figures for recovered plague lesions prior to December 1908 are not reliable.



lesions to the plague bacillus. The rats which have been classed as sub-acute plague, in our Bombay observations, have been distributed in Poona between the acute and resolving groups. The criteria as to what constituted a scar in the spleen or what degree of thickening of the capsule of the spleen entitled the condition to be called perisplenitis differed considerably in Poona and Bombay, the observations being in the hands of different workers in the two places. The same difficulty in classifying the rats was not however experienced in regard to adhesions of the spleen to surrounding structures. Here a very definite fact had to be ascertained so that there was no variation in the grouping in this case in the two places or in the same place when the observations were made over a period of years. For this reason adhesions between the spleen and surrounding structures only have been shown in the group of resolving lesions in table III and the chart, which detail the number of rats examined each month in Poona for the whole period of observations, which extended to nearly four years. This table and chart clearly show that the pathological conditions which we have called resolving and resolved plague, in spite of the fact that we were often unable to obtain absolute proof that they had been produced by the plague bacillus by isolating that organism from them, occur most frequently during and immediately after a plague epizootic<sup>1</sup>. Nevertheless there is ample evidence to show that sometimes lesions very similar in appearance to those produced by the plague bacillus are brought about by some cause as yet unknown to us for these lesions continue to occur, it is true with diminished frequency, even when plague has not been epizootic for years. Moreover, as will be seen in tables IV to VIII, even young rats showed these lesions, and they could not have been exposed to plague infection for they must have been born at a time when no plague was present in the city. Table IX shows how far it was possible to prove that the lesions we have called resolving plague were due to the plague bacillus by isolating that organism from them. In this table the so-called resolving plague lesions have been divided into two groups, visceral and peripheral; the former includes abscesses and necrotic areas in the spleen and liver while in the latter are grouped all buboes and abscesses in the lymphatic glands. It will be observed that only a small number (17 %) of resolving

<sup>1</sup> These observations may throw important light on the length of life of *rattus* in nature. Assuming that adhesions do not tend to shorten the animal's life and, which is more problematical, that they do not disappear, it is clear that the absence of any excess of such lesions about a year after a plague epidemic involves the death within that period of the bulk of the rat population alive at the time of the epidemic.

lesions could be shown to contain living plague bacilli while, from the very nature of the lesions, it was not possible to demonstrate the presence of plague bacilli in the resolved lesions. Attention has to be drawn to two confirmed rats with visceral lesions in June and August 1909. From the age of the rats, as judged by their weight, the character of the lesions and the circumstances in which the rats were found we have reason to think that these had been infected some time towards the close of the first epizootic, which, as far as we could obtain evidence,

TABLE IV.

*Incidence of glandular lesions (abscesses as well as necrotic areas) according to the age of the rat in quarterly periods from the commencement of observations in Poona.*

Quarter ending	Young <i>rattus</i>			Adult <i>rattus</i>			Total <i>rattus</i> (adult and young)		
	<i>Rattus</i> examined	No. with glandular lesions	Percentage	<i>Rattus</i> examined	No. with glandular lesions	Percentage	<i>Rattus</i> examined	No. with glandular lesions	Percentage
December 1908*	2963	8	0·27	3799	43	1·13	6762	51	0·75
March 1909	3673	3	0·08	3914	41	1·05	7587	44	0·58
June "	4478	1	0·02	3485	6	0·17	7963	7	0·09
September "	5638	3	0·05	5193	13	0·25	10831	16	0·15
December "	5443	5	0·09	6446	12	0·19	11889	17	0·14
March 1910	4336	1	0·02	6266	9	0·10	10602	10	0·09
June "	4041	3	0·07	4886	5	0·10	8927	8	0·09
September "	4152	2	0·05	5234	10	0·19	9386	12	0·13
December "	5999	2	0·04	6335	17	0·27	12334	19	0·15
March 1911	3704	2	0·05	5895	5	0·08	9599	7	0·07
June "	3599	2	0·06	4009	4	0·10	7608	6	0·08
September "	3936	0	—	4163	2	0·05	8099	2	0·02
December "	2589	6	0·23	3605	32	0·89	6194	38	0·61
March 1912	1582	4	0·25	2948	38	1·29	4530	42	0·93
Totals	56133	42	0·07	66178	237	0·36	122311	279	0·23

\* Figures for November and December only, those prior to November not available and figures for November not reliable.

came to an end about the month of March, so that these rats had harboured plague bacilli in their bodies for from three to five months. Both rats had abscesses in the spleen. Attention has also to be drawn to three rats caught in November 1909 which showed cervical buboes from which the plague bacillus was isolated. These three rats were associated with one of the limited epizootics which we mentioned occurred during the plague-free interval. The affected rats were young rats, not three months old, so that they must have acquired infection



TABLE V.

*Incidence of splenic lesions (abscesses as well as necrotic areas) according to the age of the rat in quarterly periods from the commencement of observation in Poona.*

Quarter ending	Young <i>rattus</i> less than 70 grammes			Adult <i>rattus</i> 70 grammes and over			Total <i>rattus</i> (adult and young)		
	<i>Rattus</i> examined	No. with splenic abscesses	Percentage	<i>Rattus</i> examined	No. with splenic abscesses	Percentage	<i>Rattus</i> examined	No. with splenic abscesses	Percentage
December 1908 *	2963	13 (a)	0.44	3799	42 (b)	1.11	6762	55	0.81
March 1909	3673	2	0.05	3914	26 (c)	0.66	7587	28	0.37
June "	4478	1	0.02	3485	6	0.17	7963	7	0.09
September "	5638	3	0.05	5193	6	0.12	10,831	9	0.08
December "	5443	0	—	6446	2	0.03	1889	2	0.02
March 1910	4336	0	—	6266	3	0.05	10,602	3	0.03
June "	4041	1	0.02	4886	2	0.04	8927	3	0.03
September "	4152	7	0.17	5234	12	0.23	9386	19	0.20
December "	5999	9	0.15	6335	17	0.27	12,334	26	0.21
March 1911	3704	0	—	5895	3	0.05	9599	3	0.03
June "	3599	1	0.03	4009	2	0.05	7608	3	0.04
September "	3936	3	0.08	4163	0	—	8099	3	0.04
December "	2589	19 (d)	0.73	3605	29 (e)	0.80	6194	48	0.77
March 1912	1582	7 (f)	0.44	2948	31 (g)	1.05	4530	38	0.84
Totals.....	56,133	66	0.12	66,178	181	0.27	122,311	247	0.20

\* Figures for November and December only, those prior to November not available and figures for November not reliable.

(a) One *rattus* with combined visceral and peripheral lesions included amongst visceral and not amongst peripheral.

(b) Eight *rattus* with combined visceral and peripheral lesions included amongst visceral and not amongst peripheral types.

(c) Four *rattus* with combined visceral and peripheral lesions included amongst visceral and not amongst peripheral types.

(d) Four *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

(e) Ten *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

(f) Three *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

(g) Thirteen *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

at some date after March 1909, when we believe the first epizootic terminated. Excepting the rats mentioned above, none were found harbouring living plague bacilli during the plague-free interval, although a most careful examination by animal, cultural and microscopical tests was made of every suspected rat. Despite this fact, however, we have

TABLE VI.

*Splenic scars in quarterly periods since commencement of observations in Poona. Young and adult rattus shown separately.*

Quarter ending	Young <i>rattus</i>			Adult <i>rattus</i>			Total <i>rattus</i> (adult and young)		
	<i>Rattus</i> examined	No. with scars	Percentage	<i>Rattus</i> examined	No. with scars	Percentage	<i>Rattus</i> examined	No. with scars	Percentage
December 1908*	2963	3	0·10	3799	22	0·58	6762	25	0·37
March 1909	3673	14	0·38	3914	53	1·35	7587	67	0·88
June "	4478	9	0·20	3485	60	1·72	7963	69	0·87
September "	5638	38	0·67	5193	89	1·71	10831	127	1·17
December "	5443	36	0·66	6446	118	1·83	11889	154	1·30
March 1910	4336	8	0·18	6266	90	1·44	10602	98	0·92
June "	4041	25	0·62	4886	67	1·37	8927	92	1·03
September "	4152	36	0·87	5234	133	2·54	9386	169	1·80
December "	5999	66	1·10	6335	200	3·16	12334	266	2·16
March 1911	3704	42	1·13	5895	116	1·97	9599	156	1·65
June "	3599	28	0·78	4009	83	2·07	7608	111	1·46
September "	3936	52	1·32	4163	109	2·62	8099	161	1·99
December "	2589	47	1·82	3605	123	3·41	6194	170	2·74
March "	1582	7	0·44	2948	78	2·65	4530	85	1·88
Totals	56133	411	0·73	66178	1341	2·03	122311	1750	1·43

\* Figures for November and December only, those prior to November not available and figures for November not reliable.

little doubt that many of the lesions we found were primarily produced by the plague bacillus. It cannot be denied, however, as has been frequently stated above, that some of the lesions were produced by other unknown cause or causes so that the classification "resolving" and "resolved" plague is to that extent misleading.

TABLE VII.

*Perisplenitis in quarterly periods since the commencement of observations in Poona.*

Quarter ending	Young <i>rattus</i>			Adult <i>rattus</i>			Total <i>rattus</i> (adult and young)		
	<i>Rattus</i> examined	No. with perisplenitis	Percentage	<i>Rattus</i> examined	No. with perisplenitis	Percentage	<i>Rattus</i> examined	No. with perisplenitis	Percentage
December 1908*	2963	5	0·17	3799	35	0·92	6762	40	0·59
March 1909	3673	10	0·27	3914	101	2·58	7587	111	1·46
June "	4478	5	0·11	3485	42	1·20	7963	47	0·59
September "	5638	5	0·09	5193	38	0·73	10,831	43	0·40
December "	5443	0	—	6446	31	0·48	11,889	31	0·26
March 1910	4336	0	—	6266	34	0·54	10,602	34	0·32
June "	4041	0	—	4886	25	0·51	8927	25	0·28
September "	4152	5	0·12	5234	41	0·78	9386	46	0·49
December "	5999	1	0·02	6335	33	0·52	12,334	34	0·28
March 1911	3704	1	0·03	5895	39	0·66	9599	40	0·42
June "	3599	1	0·03	4009	25	0·62	7608	26	0·34
September "	3936	6	0·15	4163	37	0·89	8099	43	0·53
December "	2589	12	0·46	3605	33	0·92	6194	45	0·73
March 1912	1582	2	0·13	2948	42	1·42	4530	44	0·97
Totals ...	56,133	53	0·09	66,178	556	0·84	122,311	609	0·50

\* Figures for November and December only, those prior to November not available and figures for November not reliable.

TABLE VIII.

*Adhesions of spleen in quarterly periods since commencement of observations in Poona.*

Quarter ending	Young <i>rattus</i>			Adult <i>rattus</i>			Total <i>rattus</i> (adult and young)		
	<i>Rattus</i> examined	No. with adhesions	Percentage	<i>Rattus</i> examined	No. with adhesions	Percentage	<i>Rattus</i> examined	No. with adhesions	Percentage
December 1908*	2963	1	0·03	3799	28	0·74	6762	29	0·43
March 1909	3673	9	0·25	3914	138	3·53	7587	147	1·94
June "	4478	0	—	3485	69	1·98	7963	69	0·87
September "	5638	6	0·11	5193	74	1·42	10831	80	0·74
December "	5443	6	0·11	6446	51	0·79	11889	57	0·48
March 1910	4336	2	0·04	6266	23	0·37	10602	25	0·24
June "	4041	0	—	4886	12	0·24	8927	12	0·13
September "	4152	1	0·02	5234	21	0·40	9386	22	0·23
December "	5999	6	0·10	6335	20	0·32	12334	26	0·21
March 1911	3704	2	0·05	5895	39	0·66	9599	41	0·43
June "	3599	2	0·06	4009	22	0·55	7608	24	0·32
September "	3936	1	0·03	4163	17	0·41	8099	18	0·22
December "	2589	7	0·27	3605	41	1·14	6194	48	0·77
January 1912	1582	2	0·13	2948	80	2·71	4530	82	1·81
	56133	45	0·08	66178	635	0·96	122311	680	0·56

\* Figures for November and December only, those prior to November not available and figures for November not reliable.

TABLE IX.

*Table showing the sensational prevalence of plague and so-called post plague lesions amongst rattus of regular (Poona City) trapping in monthly periods from June 1908 to March 1912.*

Months	Total alive rattus examined	Acute plague		Resolving plague				Recovered plague		
		Dead	Alive	Confirmed	Not con- firmed	Confirmed	Not con- firmed	Adhesions	Scars	Peri- splenitis
June 1908	3977	—	—	—	2	—	1	—	—	—
July "	5351	—	—	—	—	—	4	—	—	—
August "	5946	—	—	—	1	—	3	—	—	—
September "	6115	24	10	2	—	2	14	—	—	—
October "	4913	36	29	3	14	3	7	—	—	—
* November "	3814	5	30	5	14	3	7	3	3	3
December "	2923	1	27	3	34	7	33	26	22	37
January 1909	2376	2	6	1	13	4	15	43	24	55
February "	2284	1	1	2	8	2	10	57	17	30
March "	2906	—	—	—	5	—	13	48	26	22
April "	2644	—	—	—	4	—	1	28	12	14
May "	2479	—	—	—	2	—	3	19	25	15
June "	2830	—	—	1†	—	—	3	22	32	18
July "	3727	—	—	—	2	—	5	31	42	19
August "	3554	—	—	1‡	3	—	2	24	31	11
September "	3520	—	—	—	4	—	9	25	54	13
October "	3508	—	—	—	1	—	4	21	33	3
November "	4059	—	—	—	1	3§	4	21	58	10
December "	4306	—	—	—	1	—	6	15	63	18
January 1910	3462	—	—	—	1	—	6	12	37	8
February "	3659	—	—	—	2	—	1	6	35	10
March "	3273	—	—	—	1	—	3	7	26	16
April "	3120	—	—	—	—	—	1	3	29	7
May "	2875	—	—	—	1	—	2	2	22	8
June "	2916	—	—	—	2	—	5	7	41	10
July "	3140	—	—	—	4	—	6	8	52	14
August "	3534	—	—	—	13	—	4	7	61	17
September "	2712	—	—	—	2	—	2	7	56	15
October "	3628	—	—	—	8	—	3	9	85	11
November "	4408	—	—	—	14	—	8	8	92	9
December "	4298	—	—	—	4	—	8	9	89	14
January 1911	3380	—	—	—	1	—	3	11	56	14
February "	2929	—	—	—	1	—	2	13	56	13
March "	3290	—	—	—	1	—	2	17	46	13
April "	2775	—	—	—	2	—	3	13	37	11
May "	2622	—	—	—	—	—	2	5	41	8
June "	2211	—	—	—	1	—	1	6	33	7
July "	2703	—	—	—	—	—	—	11	42	13
August "	2631	—	—	—	—	—	—	6	61	13
September "	2765	1	8	—	3	—	2	1	58	17
October "	1840	8	15	1	8	—	4	10	48¶	10
November "	2401	12	12	5	12	2	9	11	64	18
December "	1953	9	4	6	16	12	11	27	58	17
January 1912	1802	3	5	8	14	15	12	21	38	18
February "	1527	3	0	2	7	6	6	33	24	20
March "	1201	—	—	2	5	1	2	28	23	6

\* Figures for post plague lesions not reliable before November 1908.

† This rat was caught alive at Raviwar 174 on June 7, 1909 (wt. 130 grms.).

‡ This rat was caught alive at Bhowanee 1004 on Aug. 19, 1909 (wt. 170 grms.).

§ (1) Caught alive at 179 Nana on Nov. 3, 1909 (wt. 85 grms.). (2) Caught alive at 186 Nana on Nov. 13, 1909 (wt. 52 grms.). (3) Caught alive at 186 Nana on Nov. 13, 1909 (wt. 80 grms.).

|| Three of these were either scars or perisplenitis.

¶ One of these was either scar or perisplenitis.

III. *The examination of Madras rats.*

We propose now to support this latter contention by detailing the result of the examination of 15,523 rats caught alive in Madras city, which has been free from plague since 1905<sup>1</sup>. Table X shows for each month during the year 1910 the number of rats examined and those found with lesions which might have been classed under the terms "resolving" or "resolved" plague as defined above but which could not have been produced by the plague bacillus. In no case was any organism resembling the plague bacillus found. In all lesions were found in 0.23 % of the rats examined.

TABLE X.

Month	Number of rats examined	Number with visceral lesions	Number with peripheral lesions
January	3151	2	0
February	1978	2	0
March	1197	2	1
April	1011	4	0
May	1502	2	1
June	1331	1	1
July	1007	3	2
August	899	0	2
September	965	3	0
October	1132	4	1
November	1021	3	0
December	329	1	0
Totals	15523	27	8

*List of Madras rats with lesions resembling resolving and resolved plague lesions.*

1910		
5551	Jan. 10	Perisplenitis with adhesions
5726	„ 11	Perisplenitis with adhesions to omentum.
8079	Feb. 5	Single chronic abscess in the spleen.
8914	„ 12	Perisplenitis.
9616	Mar. 3	Left kidney atrophied to half size adherent to spleen.
9770	„ 5	Submaxillary abscess with inspissated pus; not plague.
10646	„ 30	Perisplenitis; spleen adherent to kidney.
10789	April 2	Perisplenitis; spleen adherent to abdominal wall.
11134	„ 12	Solitary chronic abscess of spleen; no evidence of plague.
11191	„ 23	Spleen constricted and adherent to abdominal wall.
11175	„ 23	Several necrotic areas in spleen; adherent abdominal wall.
13010	May 28	Left inguinal abscess; no evidence of plague.
13026	„ 28	Adhesions between spleen and abdominal wall.
13347	„ 7	Liver and spleen adherent to the stomach.
14303	June 23	Submaxillary abscess; no evidence of plague.
14486	„ 25	Liver, spleen and intestines matted together by strong fibrous adhesions; spleen adherent to abdominal wall, a chronic abscess in liver with thick yellow pus.

<sup>1</sup> See above, p. 209.

14672	July 5	Spleen, liver and intestines matted together; spleen adherent to abdominal wall; abscesses in liver; not plague.
14811	„ 11	Right inguinal abscess; not plague.
14842	„ 12	Spleen adherent to abdominal wall.
15009	„ 19	Spleen adherent to abdominal wall.
15095	„ 21	Right axillary abscess with thick cheesy pus; not plague.
15828	Aug. 12	Submaxillary abscess; not plague.
15939	„ 15	Submaxillary abscess with cheesy pus.
16454	Sept. 1	Perisplenitis, spleen adherent to abdominal wall.
16900	„ 17	Spleen adherent to abdominal wall and kidney.
17092	„ 23	Bisected spleen.
17700	Oct. 14	A small area of perisplenitis.
17767	„ 15	Spleen adherent to abdominal wall.
18013	„ 19	Left submaxillary abscess.
18149	„ 21	Spleen adherent to abdominal wall and kidney.
18360	„ 26	Adhesion between spleen and abdominal wall.
18763	Nov. 5	Liver and spleen adherent to the stomach.
19223	„ 19	Spleen adherent to the stomach and the abdominal wall.
19297	„ 21	Spleen adherent to the stomach and abdominal wall.
19898	Dec. 9	Lower portion of the spleen replaced by fibrous tissue and adherent to the kidney.

*Conclusions.*

1. Lesions, for which we propose the terms resolving and resolved plague lesions, are found in rats which have been subjected to plague infection and in certain individuals are stages in the natural process of recovery from the acute form of this disease.

2. These lesions are very different from the lesions found in the acute stages of the disease. They are generally localised and confined for the most part to the spleen.

3. It is not always possible to prove that these lesions are due to the plague bacillus by isolating that bacillus from them, for in many cases the bacilli have been killed, have disappeared or been replaced by some other organism.

4. That the lesions are frequently produced by the plague bacillus has been shown by (a) demonstrating the presence of plague bacilli in some of these lesions which exactly resemble others, either in the same rat or in other rats, in which no plague bacilli can be found, (b) by showing that the lesions increase in numbers during and after epizootics of the disease.

5. Nevertheless the fact must be borne in mind that very similar lesions can be produced by other causes, as has been shown by our experience of the examination of rats in Madras city, where the disease is not present.

### LIII. THE EXPERIMENTAL PRODUCTION OF RESOLVING PLAGUE AND POST-PLAGUE LESIONS IN RATS.

I. IN the course of some experiments on the relative immunity to plague of rats from different parts of India, the details of which are given above (p. 229), a large number of rats were inoculated with varying small doses of plague bacilli. The doses given were quite small and a considerable number of the rats inoculated survived. Of these survivors a certain percentage showed lesions similar to what we have described (above, p. 266) as resolving plague and post-plague lesions in naturally infected rats. In all, 3600 rats were inoculated and of these 1079 survived. The survivors were killed by chloroform three weeks after inoculation and examined. 119 or 11 % of these showed resolving plague or post-plague lesions.

Table I shows the number of rats from each source, the numbers and percentages of survivors and of survivors showing resolving and post-plague lesions.

It will be seen that, excluding the Bhagalpur and Yeotgaon figures which are based on a single experiment, the rats showing the greatest susceptibility to plague also show the largest number of resolving plague lesions among the survivors. That is, in addition to the rats which have died of plague a considerable number of the remainder have passed through a moderately severe attack which has left well-marked signs behind it. Thus of 1000 Madras rats inoculated, 9 of the 18 survivors showed resolving lesions, only 9 rats showing no sign of having passed through an attack of plague. The converse also holds true, the rats which are very resistant to plague showing only a small percentage of resolving lesions among the survivors. For example, out of 300 young Poona rats, 160 survived and 5 only of these showed resolving plague lesions, the remaining 155 showing no sign of having passed through an attack.

Wild rats such as those used in these experiments are of course liable to have chronic plague when inoculated. In the preceding paper the data are given on this point as regards Madras, Bombay and Poona. 1000 Madras rats would probably include 2 rats with post-plague lesions, and an average sample of 700 Bombay or Poona rats

about 6 or 8 animals with resolving and recovered plague. Even if all these survived the test inoculations, they will not account for the considerable number actually found.

TABLE I.

			Number inoculated	Number of survivors	Percentage of survivors	Number of survivors with resolving and post-plague lesions	Percentage of survivors with resolving and post-plague lesions
Madras rats	...	...	1000	18	2	9	50
Dacca rats	...	...	200	10	5	3	30
Kirkee rats	...	...	200	61	30	19	31
Adult Bombay <i>rattus</i>	...	...	700	287	41	24	8
Young Bombay <i>rattus</i>	...	...	300	127	42	5	4
Adult Poona rats	...	...	700	344	49	46	13
Young Poona rats	...	...	300	160	53	5	3
Bhagalpur rats	...	...	100	43	43	7	16
Yeotgaon rats	...	...	100	29	29	1	3

The following lesions were found :—

Lesion	Number of cases
Pelvic buboes	28
Abscesses in spleen	55
„ liver	6
„ kidney	1
Necrotic areas in spleen	10
Adhesions of spleen to abdominal wall	30
„ „ omentum	9
„ „ kidney	3
„ „ stomach	1
„ „ ovary	1
Scars in spleen	11
Perisplenitis	17

The lesions were single in 83 cases and multiple in 36, two or more lesions, such as abscesses in the spleen and adhesion to the abdominal wall, being associated in many cases.

#### *Resolving Plague Lesions.*

*Buboes*:—28 cases. These were pelvic in situation, the injection being always given in the groin. They were in all respects similar to the chronic buboes found in wild rats.

*Abscesses in the spleen*:—55 cases. These varied in number from 1 to 30 or 40 and in size from a pinhead to a small pea. 1 larger abscess about the size of a cherry was found associated with 2 of the typical small abscesses.



Adhesions between the surfaces of the abscesses and the surrounding structures were present in 26 cases as under:—

Adhesion to abdominal wall	...	...	...	16
„ omentum	...	...	...	3
„ omentum and abdominal wall	...	...	...	3
„ kidney	...	...	...	1
„ kidney and abdominal wall	...	...	...	2
„ abdominal wall and ovary	...	...	...	1

Scars in the spleen and perisplenitis were associated with abscesses in a few cases.

*Abscesses in Liver*:—6 cases. In 4 cases there were also abscesses in the spleen and adhesions of the spleen to surrounding structures. The liver abscesses resembled those in the spleen in appearance.

*Necrotic areas in the spleen*:—10 cases. In 5 out of the 10 cases these areas were small spots of well-defined appearance, well marked off from the surrounding tissues, and unassociated with other lesions. In one case necrotic spots of this sort were present along with abscesses in the spleen, and in another along with adhesion to the abdominal wall. In the remaining 3 cases the necrotic areas were of the “band” or wedge type and were associated with other lesions as follows:—

1. Necrotic band + adhesion to omentum.
2. Necrotic band + necrotic wedge + scar + adhesion to abdominal wall and stomach.
3. Necrotic band + adhesion to abdominal wall and omentum + abscesses in the spleen.

#### *Post-Plague Lesions.*

*Adhesions between the spleen and neighbouring structures*:—37 cases. The adhesions were with a single structure in 30 cases and with more than one structure in 7 cases. The order of frequency was the same as in naturally infected rats, adhesion with the abdominal wall being commonest and adhesion with the omentum next in frequency.

The following were the lesions in the spleen found associated with adhesions:—

	...	...	...	Cases
Abscesses in spleen	...	...	...	25
Abscesses and necrotic areas in spleen	...	...	...	1
Necrotic areas in spleen	...	...	...	4
Perisplenitis	...	...	...	3
Scars	...	...	...	2
No other lesion	...	...	...	2
Total	...	...	...	37

It will be seen that in the great majority of cases (about 95 %) the adhesions were associated with conditions which were obviously due to a recent acute attack of plague. The adhesions, when associated with abscesses, always commenced directly from the surface of the abscesses and were short and friable and of recent formation. When associated with necrotic areas they were as a rule in the form of matting rather than definite bands. When associated with perisplenitis or scars only or when no other lesion was associated, the adhesions were longer and firmer and formed well-organised tough bands.

*Perisplenitis*:—17 cases. This was usually in the form of small well-defined surface patches of a glistening pearly white appearance. In several cases it was associated with adhesions of the spleen to surrounding structures.

All these lesions exactly resemble those we have found in naturally infected rats and the circumstances of their occurrence show clearly that they are stages on the way to recovery from an attack of plague of moderate severity.

II. During the attempt to measure any seasonal variation in immunity of the rats at Poona which was made during 1908-9 (vol. x. p. 523, and *supra*, p. 229), in all 3800 rats were inoculated in the right thigh with a small dose of spleen emulsion. Of these 371 died of causes other than plague, 1321 died of acute plague and 2108 survived for 21 days when they were killed with chloroform and examined. At that time our views on resolving plague were undeveloped. We have, however, a record of the occurrence of encapsuled abscesses and some other details which are of interest. Abscesses containing plague-like organisms, the contents of which gave plague to experimental animals in the great majority of cases in which the test was used, were found as follows, the lesions in 31 cases being multiple.

	Right	Left	Both	Total
Submaxillary and cervical glands ...	7	6	5	18
Axillary glands ... ..	6	3	0	9
Pelvic glands ... ..	227	2	3	232
Inguinal glands ... ..	33	2	0	35
Total glandular abscesses ...	...	...	...	294
Liver ... ..	...	...	...	8
Spleen ... ..	...	...	...	18

Altogether 296 animals out of 2108 (14.1 %) had abscesses; visceral abscesses were noted in only 26 cases (1.2 %). As has been shown

above (p. 276), such abscesses would be expected in about 5 per thousand of wild Poona *rattus* or about 20 in the original 3800 animals. Evidently, therefore, the lesions were mostly produced by the experimental inoculation.

The distribution of the buboes in the 1321 rats dying of acute plague may be compared with the distribution of the glandular abscesses: the two series give very similar results:—

			Acute plague buboes	Chronic abscesses
Submaxillary	...		1·3 %	4·5 %
Axillary	...	...	0·4	1·4
Pelvic	...	...	79·7	74·9
Inguinal	...	...	3·4	8·5
Multiple	...	...	15·2	10·7
Total	...	...	100·0	100·0

To give some idea of the time relations of these processes we give also the proportion of the inoculated rats dying at various periods after inoculation:

1st day ...	...	...	nil	
2nd	...	...	12	per thousand
3rd	...	...	285	
4th	...	...	287	
5th	...	...	156	
6th	...	...	103	
7th	...	...	69	1st week 912 per thousand
8th	...	...	35	2nd week 74 „
9th	...	...	11	3rd week 14 „
10th	...	...	6	
11th	...	...	11	
12th	...	...	5	
13th	...	...	4	
14th	...	...	2	
15th to 21st days	...	...	14	

## LIV. EXPERIMENTAL PLAGUE EPIDEMICS AMONG RATS.

(Fourth Communication.)

IN the last report (vol. x. p. 315) the details of five experimental epizootics with *Mus rattus* were given. The data of three more are now available and these eight experiments together form a series extending over a complete year from September 1909.

The experiments may be summarised as follows:—

*Experiment I. Sept. 24th to Nov. 2nd, 1909.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	10	9	15	1	0	1 found on Sept. 27 contained <i>B. pestis</i> in its stomach
No. 8	10	9	15	0	0	Nil
No. 9	10	8	15	0	0	Nil
No. 10	10	10	15	2	0	Average 2.5 per rat
No. 11	10	8	15	3	0	Average 1.4 per rat
No. 12	10	8	15	0	1	Average 7 per rat

*Experiment II. Nov. 5th to Dec. 13th, 1909.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	14	10	15	0	0	One flea found on Nov. 15
No. 8	15	13	15	0	1	One flea found on Nov. 29
No. 9	14	12	15	0	0	Nil
No. 10	10	10	15	5	0	Average 13 per rat
No. 11	10	9	15	5	4	Average 18 per rat
No. 12	10	10	15	7	0	Average 39 per rat

*Experiment III. Dec. 21st, 1909, to Jan. 24th, 1910.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	5	5	15	0	0	Godown flea infested on Jan. 17
No. 8	5	5	15	0	1	Nil
No. 9	5	4	15	0	2	Nil
No. 10	5	5	15	10	1	Average 34 per rat
No. 11	5	5	15	6	0	Average 29 per rat
No. 12	5	5	15	11	0	Average 48 per rat

*Experiment IV. Feb. 3rd to March 10th, 1910.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	10	8	15	0	0	Nil
No. 8	10	9	15	0	0	One flea found on March 7
No. 9	10	8	15	0	0	Two fleas found on March 7
No. 10	10	7	15	9	0	Average 20 per rat
No. 11	10	8	15	6	1	Average 14 per rat
No. 12	10	9	15	4	0	Average 15 per rat

*Experiment V. March 22nd to May 2nd, 1910.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	15	15	15	0	0	One flea found on April 25
No. 8	15	14	15	0	0	Nil
No. 9	15	14	15	0	0	Four fleas found on April 25
No. 10	5	5	15	15	0	Average 11 per rat
No. 11	15	15	15	12	0	Average 30 per rat
No. 12	15	13	15	1	0	Average 21 per rat

...

*Experiment VI. May 10th to June 13th, 1910.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	5	5	15	0	0	Nil
No. 8	5	5	15	0	0	Nil
No. 9	5	4	15	0	0	Nil
No. 10	5	5	15	6	1	Average 10·3 per rat
No. 11	5	5	15	14	0	Average 15·3 per rat
No. 12	5	5	15	10	0	Average 16 per rat

*Experiment VII. June 25th to August 1st, 1910.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	10	6	15	0	0	Nil
No. 8	10	8	15	0	0	Two fleas found on August 1
No. 9	10	8	15	0	0	Nil
No. 10	5	5	15	3	2	Average 14 per rat
No. 11	5	5	15	10	0	Average 20·7 per rat
No. 12	5	5	15	11	0	Average 32 per rat

*Experiment VIII. August 15th to September 26th, 1910.*

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	10	8	15	0	0	Nil
No. 8	10	8	15	0	0	Nil
No. 9	10	6	15	0	0	Nil
No. 10	10	9	15	3	0	Average 14 per rat
No. 11	10	8	15	6	1	Average 18 per rat
No. 12	10	9	15	7	0	Average 17 per rat

The whole series of eight experiments are shortly summed up in the following table :

TABLE I.

Number	Date, 1909-10	Control godowns (7, 8, 9)		Flea godowns (10, 11, 12)	
		Rats died=per cent.	Fleas	Rats died=per cent.	Fleas per rat
I	24th Sept.—2nd Nov.	1/45 = 2%	1 found	5/45 = 11%	4
II	5th Nov.—13th Dec.	0/45	2 found	17/45 = 38	23
III	21st Dec.—24th Jan.	0/45	present at end	27/45 = 60	37
IV	3rd Feb.—10th Mar.	0/45	3 found	19/45 = 42	16
V	22nd Mar.—2nd May	0/45	none	28/45 = 62	21
VI	10th May—13th June	0/45	none	30/45 = 67	14
VII	25th June—1st Aug.	0/45	2 found	24/45 = 53	22
VIII	15th Aug.—26th Sept.	0/45	none	16/45 = 36	16
Totals	...	1/360 = 0.3%		166/360 = 46%	

These experiments fully confirm those already published as to the predominant influence of the presence of fleas on the spread of plague infection from the infected rats put into the godowns to their healthy companions. In the present communication we desire to discuss the variations in the severity of the epizootics in the flea-infected godowns in the different experiments.

1. *Flea prevalence.* The flea population in the godowns was produced by the introduction of fleas removed from wild rats, a hundred or so being put into each godown every few days throughout the first epizootic and during the first half of the second experiment. Most of the fleas introduced in this way appear to die very soon, possibly on account of the handling involved and exposure to the sun during the process of collection, and it is some time before breeding is well established and a strong flea population available. It will be seen that there were relatively few fleas present during the first experiment, an increase during the second and a large rise during the third. Thereafter the flea prevalence fell somewhat and remained fairly constant. The fleas in the godowns were to some extent subject to

unnatural interference from the removal of infected rats, the presence of only a small number of rats in the intervals between the epizootics and the partial flooding of the floors of the godowns during the rainy months (June, July).

The data show that, with the exception of the fifth and sixth experiments, there is a very fair relationship between the flea prevalence and the number of rats dying from plague. This is especially well shown in the correspondence between the increasing severity of the first three epizootics and the rising number of fleas as breeding became established. The fifth and sixth epizootics differ from the others in that the mortality from plague is abnormally high in proportion to the flea prevalence. When, also, individual godowns are considered, the correspondence is not very close. Thus in godown 10, Experiments V and VI show a relatively excessive mortality: this is also seen in the sixth epizootic in godowns 11 and 12. In the fifth experiment the epizootic in godown 12 almost entirely failed though plenty of fleas were present.

2. *Septicaemia in the infecting rats.* The rats inoculated with plague which were introduced into each godown to start the infection were examined as soon as possible after death. Heart-blood smears were examined and the rats classified into three groups: (1) "low" when only an occasional bacillus was found in every few fields, (2) "moderate" when several bacilli were present in each field, and (3) "high" when every field showed large numbers of plague bacilli. Table II shows the results.

TABLE II.

*Table showing comparative degree of septicaemia in the inoculated rats dying in the successive epizootics.*

	1st epizootic	2nd epizootic	3rd epizootic	4th epizootic	5th epizootic	6th epizootic	7th epizootic	8th epizootic
Godown 10	Moderate	Low	High	Low	High	High	Low	Moderate
Godown 11	Low	Moderate	Moderate	Moderate	High	High	High	High
Godown 12	Low	Moderate	High	Low	High	High	Low	Moderate

Assigning the arbitrary values 1, 10 and 100 to the "low," "moderate" and "high" septicaemias respectively, we obtain the following rough numerical comparison between the average degree of septicaemia and the mortality.

TABLE III.

Number	Percent. mortality	Flea prevalence	Septicaemia
I	11	4	12
II	38	23	21
III	60	37	210
IV	42	16	12
V	62	21	300
VI	67	14	300
VII	53	22	102
VIII	36	16	120

The importance of the degree of septicaemia in the rats from which the fleas derive infection is obvious and its quantitative bearings on the possibility of transmission have been previously pointed out (vol. VII. p. 395, vol. VIII. pp. 286, 293). It has also been shown (vol. VI. p. 521) that rats killed during the later stages of the disease show an enormous variation in the degree of septicaemia; while the extreme variations in average flea prevalence in the different experiments are 4 and 37, the variation in the abundance of bacilli in the blood of the infecting rats may well be ten times as great. Be that however as it may, the fact remains that the highest average degree of septicaemia was found in experiments V and VI, where the mortality was high and the flea prevalence relatively low. Taking all the experiments in each godown separately, it appears that when the septicaemia was "low" 35 out of 105 (33 %) rats died in 7 epizootics, with "moderate" septicaemia 36 out of 105 (34 %) and with "high" septicaemia 95 out of 150 (63 %). It seems clear, therefore, that, though all the transmitting fleas do not in these experiments necessarily derive their infection from the inoculated rats put into the godowns, the observed differences in the degree of septicaemia afford some explanation of the varying severity of the different epizootics.

3. *Climatic conditions.* Continuous readings of temperature and humidity were kept in the godowns by means of thermographs and hygrometers. The temperature corresponds in some degree with the outside temperature in Bombay but the humidity throughout tends to be a good deal higher than the outside readings. This is due to the fact that the godowns were small and rather badly ventilated and, being lined with cement, they retained any moisture that got in during rainy weather.



Table IV shows the maximum, minimum and mean weekly mean temperature and humidity inside the godowns during the experiment.

TABLE IV.

Experiment	Temperature	Humidity	Flea prevalence	Rat mortality %
I	76—80 : 78° F.	83—96 : 89% satn.	4	11
II	72—78 : 75° „	70—85 : 76 „	23	38
III	67—73 : 71° „	72—88 : 81 „	37	60
IV	70—75 : 72° „	71—78 : 75 „	16	42
V	81—84 : 83° „	86—94 : 88 „	21	62
VI	83—88 : 85° „	91—99 : 93 „	14	67
VII	80—82 : 81° „	89—99 : 92 „	22	53
VIII	75—79 : 78° „	90—97 : 94 „	16	36

The figures do not appear to show that the variations in temperature and humidity had a greater influence than other factors on the flea prevalence or the rat mortality<sup>1</sup>.

4. *Relation to the natural epizootic.* During the period covered by the experiments, the prevalence of natural plague among the wild rats in Bombay was above the mean level from the first week in January to the third week in May. The first three experiments were made therefore during the earliest part of the natural epizootic, and they show an increasing flea prevalence associated with an increasing rat mortality. We have, however, already indicated that in the present experiments the flea prevalence was probably conditioned during the earlier experiments by the manner in which the godowns were stocked with fleas. The severe epizootics of Experiments V, VI and VII occurred during the decline of the natural epizootic and in the beginning of the off-plague season. We have previously recorded similarly severe experimental epidemics among guinea pigs during the off-plague season (vol. VII. p. 427), though the general correspondence between season and severity was more definite than in the present series (see also vol. VIII. p. 279).

5. *Susceptibility of the rats used.* We have shown (vol. VI. p. 505, VIII. p. 333, X. p. 458) that a considerable proportion (30—70 % according to the dose) of wild Bombay *rattus* possess a high degree of resistance to infection by hypodermic inoculation. In some of the present experiments (III, V, VI) as many as 60—67 % of the rats died, and it is questionable whether any mortality in excess of this

<sup>1</sup> Compare the series discussed in vol. VIII. p. 279 where, with less complete data, high temperatures appeared to have a considerable effect in limiting the epizootics.

could be expected from flea transmission with these relatively immune animals. If that is so, once the conditions are sufficiently favourable to cause a mortality of this order, any further improvement in the facilities for the transmission of infection may lead to no corresponding increase of mortality. It should also be noted that, using 45 rats in each experiment, there is considerable room for accidental variations in mortality<sup>1</sup>.

6. *Conclusions.* As regards the mortality in the different epizootics it appears therefore that the flea prevalence and the degree of septicaemia developing in the infected rats were the chief influences at work.

*Disposal of the survivors of the epizootics.*

A. *Examination for signs of resolving plague.* The survivors in the first three experiments were used.

TABLE V.

Experiment	Control godowns			Flea godowns		
	Examined	Resolving plague	Recovered plague	Examined	Resolving plague	Recovered plague
I	33	0	0	27	0	1
II	37	0	0	21	1	2
III	37	0	0	17	1	4
Totals	107	0	0	65	2	7

Two of the resolving plague lesions were shown to contain virulent plague bacilli. Of the seven examples of recovered plague, five were perisplenitis and two scars in the spleen (see vol. x. p. 335).

Of the survivors from the flea godowns therefore about 3% showed resolving and 11% recovered plague: of 107 survivors from the control flea-free experiments none showed any signs of having had an attack of plague.

B. *Resistance of the survivors of the epizootics.* The survivors of the fourth, sixth and seventh experiments were tested for their immunity by the hypodermic inoculation of an emulsion of plague rat's spleen.

<sup>1</sup> Thus in the fifth experiment in godown 10 all of the 15 rats died, in godown 11 12 out of 15, and in godown 12 only one out of 15. The 14 survivors from the last godown were afterwards inoculated with such a dose of plague as kills about 20 per cent. of ordinary Bombay *rattus*; all of them surviving, it is legitimate to suspect that they were not a fair sample.

TABLE VI.

Experiment	Dose	Control godowns		Flea godowns	
		Inoculated	Died of plague	Inoculated	Died of plague
IV	1/5000 grm.	38	9	24	0
VI	1/5000 „	30	3	9	0
VII	1/2000 „	36	29	9	5

These figures show that the survivors among the rats which had been exposed to infection were much more immune than the control rats. In discussing the similar experiments which were previously made (vol. x. p. 332), it was pointed out that this could probably be accounted for by the elimination of most of the susceptible individuals during the experimental epizootics. In the present case this is not so clear, but there is no evidence of the rats having acquired immunity while in the godowns by the inoculation of small doses of *B. pestis* by fleas. That the immunity is only relative, is shown by the large number of deaths among the survivors of the seventh epizootic when a larger test dose of plague was given.

TABLE VII.

*Distribution of the primary bubo in rats dying of plague in the godowns (see vol. VII. p. 382).*

Cervical	...	...	...	147
Axillary	...	...	...	4
Cervical and axillary	...	...	...	1
Inguinal	...	...	...	2
Pelvic	...	...	...	2
No bubo	...	...	...	6
Eaten so that bubo could not be found	...	...	...	5
Total	...	...	...	167

## LV. OBSERVATIONS ON FLEA BREEDING IN POONA.

THE seasonal variations in the number of fleas per rat might depend either upon a variation in the number of their hosts, or a variation in the habits of the fleas themselves at different seasons of the year, or again upon the length of a flea's life at different times of the year, rather than upon the rate at which they breed. It was determined therefore to carry out these observations with a view to ascertaining what factors influence the rate of reproduction and the length of life of these insects, and to what extent any variations in their rate of reproduction or the length of their life might explain the seasonal variations in their numbers. These experiments with *X. cheopis* were commenced in July 1909 and were continued till February 1912, contemporary data as to the natural prevalence of fleas on *Mus rattus* in Poona being available throughout.

### I. EGG LAYING.

#### (a) *Egg laying at different seasons of the year.*

Fleas were caught without the assistance of chloroform on freshly caught and killed rats. A number of adult females were selected and batches of five were put into test tubes and kept in a dark place. It was a difficult and slow process but it was found afterwards that a flea could easily be transferred from one test tube to another by means of a platinum needle, for after settling on the point it seldom jumped off in transference. As little time as possible was lost between the separation of these fleas from the rat and the commencement of the observations, as it was found that the largest number of eggs were laid during the first hour after separation from the rats. After being kept in a dark box for one hour the fleas were thrown away and the number of eggs deposited in the test tube were counted.

The result of the experiment will be seen in table I which compares in periods of one month the average number of eggs laid per flea with the average 8 a.m. humidity and mean temperature for

that month from July 1909 to February 1912. These figures show that at any rate within certain limits of temperature the number of eggs laid by a flea is closely correlated to the atmospheric humidity.

TABLE I.

*Table showing the average number of eggs laid per flea (X. cheopis) in monthly periods. The average temperature and humidity in monthly periods is also shown. (Poona.)*

Month	Number of fleas	Number of eggs laid	Number of eggs per flea per hour	Average 8 o'clock humidity	Average monthly mean temperature
July 1909	145	231	1.6	84.4	75.3
Aug. „	180	423	2.4	81.9	75.9
Sept. „	225	467	2.1	82.2	75.5
Oct. „	230	457	2.0	71.8	77.0
Nov. „	255	339	1.3	55.6	74.9
Dec. „	245	331	1.4	62.3	70.8
Jan. 1910	240	317	1.3	52.1	69.7
Feb. „	198	335	1.7	39.3	72.0
March „	158	133	0.8	37.9	78.9
April „	127	79	0.6	30.3	84.5
May „	70	59	0.8	47.6	85.1
June „	111	185	1.7	73.2	79.8
July „	201	522	2.6	77.1	77.4
Aug. „	135	373	2.8	86.2	75.4
Sept. „	150	334	2.2	84.1	75.8
Oct. „	140	415	3.0	76.2	75.7
Nov. „	160	318	2.0	73.7	68.6
Dec. „	130	286	2.2	67.1	66.8
Jan. 1911	110	325	3.0	64.9	70.9
Feb. „	130	240	1.8	47.7	71.7
March „	125	187	1.5	52.5	76.8
April „	115	132	1.1	43.7	83.9
May „	120	86	0.7	52.0	87.3
June „	100	230	2.3	77.2	79.9
July „	135	411	3.0	75.6	78.4
Aug. „	130	314	2.4	85.5	74.9
Sept. „	120	335	2.8	79.9	76.4
Oct. „	95	244	2.6	75.4	81.0
Nov. „	100	204	2.0	66.9	76.4
Dec. „	90	218	2.4	62.9	71.6
Jan. 1912	70	144	2.1	57.7	71.3
Feb. „	50	101	2.0	64.0	74.3

In order to ascertain if possible the relative importance of temperature and humidity on the rate of egg laying the following experiments were carried out.

*(b) Influence of varying humidity on egg laying at room temperature.*

This experiment was carried out to ascertain the number of eggs laid in one hour after removal from rats by fully grown female fleas in atmospheres of varying humidity. The fleas were divided into two batches and put into test tubes, a plug of cotton wool was inserted and this was closed with a rubber cap. One batch of fleas was kept under ordinary atmospheric conditions at room temperature and on the cotton wool plug of the other one or two drops of water was placed. The results of this experiment are shown in table II; it will be observed that in the presence of moisture, especially in the drier months of the year, a distinctly larger number of eggs were laid by the fleas.

TABLE II.

*Table comparing the average number of eggs laid in one hour per flea in atmospheres having different degrees of humidity. (Poona.)*

Month	Average 8 o'clock humidity	Average mean tem- perature	Normal			Moistened		
			Fleas	Eggs	Average	Fleas	Eggs	Average
January 1911	64.9	70.9	25	66	2.6	10	31	3.1
February „	47.7	71.7	50	53	1.1	15	32	2.1
March „	52.5	76.8	—	—	—	—	—	—
April „	43.7	83.9	20	26	1.3	20	47	2.3
Total			95	145	1.5	45	110	2.4

*(c) Influence of varying temperature on egg laying.*

In this experiment, similar to the last, an attempt was made to ascertain the relative importance of temperature on the number of eggs laid in one hour after removal from their hosts by fully grown female fleas. In order to eliminate as far as possible the effect of relative humidity, which would vary at different temperatures, three test tubes were taken and between the rubber cap and cotton wool plug of each a piece of drying disc was placed consisting of asbestos impregnated with calcium chloride such as is used in the boxes in which photographic films are sold. These were kept for twenty-four hours in order to dry the air in the tube. The fleas were then put into these test tubes and kept in a dark place at various temperatures. One tube was kept at room temperature which varied between 24° and 28° C., another was placed in an incubator the temperature of which was from

35° to 39° C. whilst a third was kept in a box containing some ice the temperature of which was from 13° to 20° C. The results show that this variation in the temperature has a marked effect on the number of eggs laid; it will be seen in table III that the room temperature was the most favourable for egg laying and that at both the higher and the lower temperatures fewer eggs were laid.

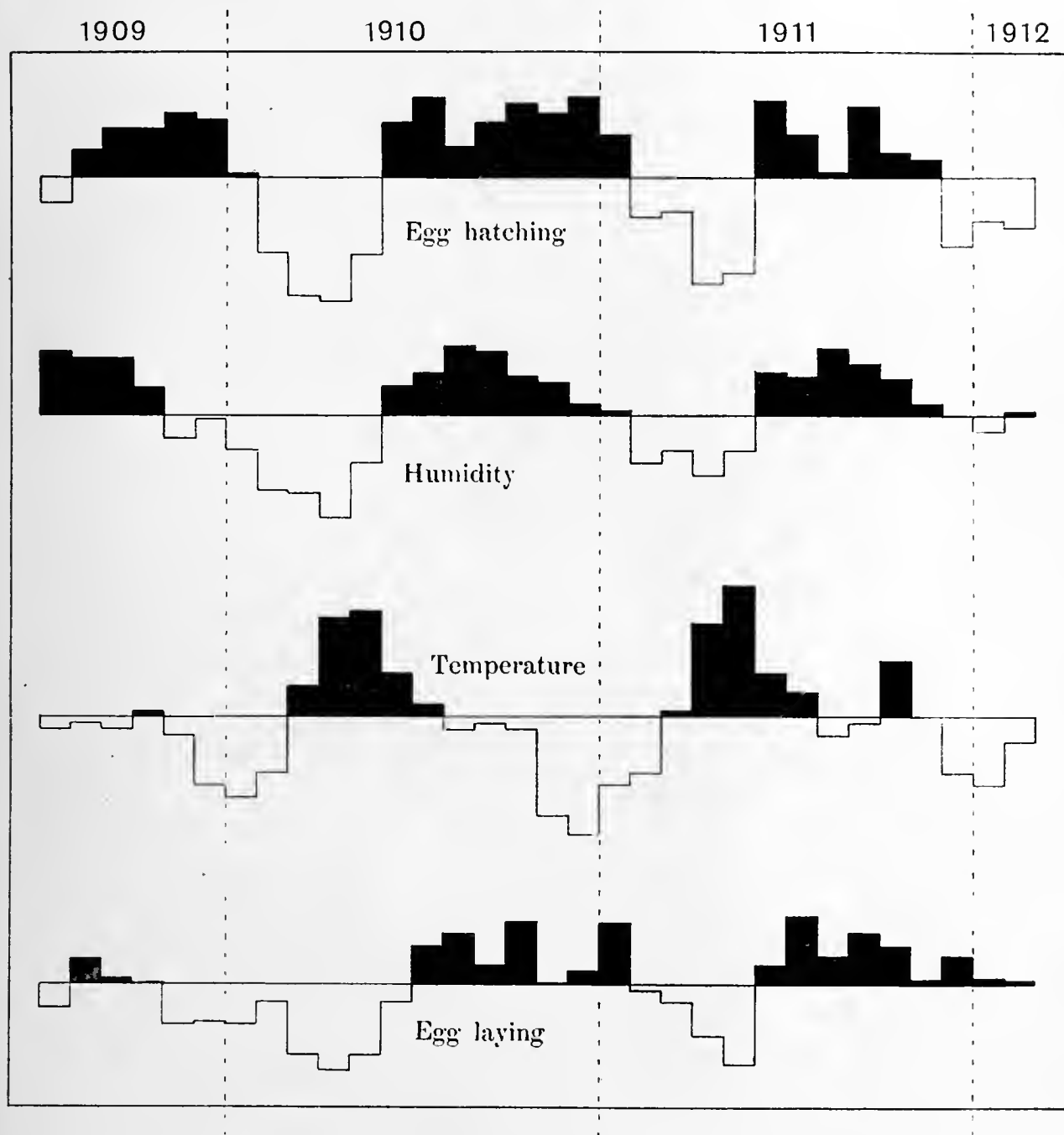


Fig. 1. The horizontal lines represent the mean values for 1910-11, and the ordinates the percentage variations for each month above and below these means.

TABLE III.

*Table showing the number of eggs laid at varying degrees of temperature when the effect of humidity has been as far as possible eliminated.*

Date	High Temperature			Room Temperature			Low Temperature			Remarks
	Mean temperature recorded	No. of fleas	No. of eggs laid	Mean temperature recorded	No. of fleas	No. of eggs laid	Mean temperature recorded	No. of fleas	No. of eggs laid	
1911-12										
Dec. 15	37	5	12	27	5	14	—	—	—	The atmosphere of the tubes was not previously rendered dry.
„ 16	35	5	9	—	—	—	20	5	5	
„ 18	38	5	3	25	5	11	20	5	11	
Jan. 1	39	5	1	—	—	—	18	5	2	Since January 1st atmosphere of the tubes was made dry prior to doing the experiment.
„ 4	38	5	—	24	5	10	14	5	—	
„ 5	39	5	—	24	5	14	—	—	—	
„ 6	37	5	—	24	5	12	13	5	—	
„ 12	—	—	—	24	5	2	14	5	—	
„ 17	39	5	—	29	5	6	15	5	—	
„ 19	39	5	—	28	5	10	15	5	—	
<hr/>										
Average number of eggs per flea since Jan. 1, 1912			0.03				1.8			
							0.07			

## II. EGG HATCHING.

### (a) Egg hatching at different seasons of the year.

Test tubes in which fleas' eggs had been deposited were placed, some in a dark box and others in an ordinary well-lighted room, but not in direct sunlight, in order to note if the number of eggs hatched was in nature affected by daylight, and in addition to ascertain the number of eggs that hatch at different seasons of the year.

The result is shown in table IV. Approximately equal numbers of flea-eggs were kept in light and dark every month throughout the period of observation. Out of 5543 kept in light and 5571 kept in dark, the percentages hatched into larvae were 32.6 and 32.9 respectively. In the table the number of eggs hatched is compared with the average 8 a.m. humidity and it appears that, at any rate within certain limits of temperature, there is a distinct correlation between the number of eggs hatched at different seasons of the year and the percentage of atmospheric humidity.



TABLE IV.

*Table showing the percentage of flea-eggs hatched in monthly periods.*

*The average monthly mean temperature and average 8 o'clock humidity are also shown for comparison.*

Month	Number of eggs observed	Number of eggs hatched	Percentage of flea- eggs hatched	Average 8 o'clock humidity	Average monthly mean temperature
July 1909	381	83	20·9	84·4	75·3
Aug. "	454	145	31·9	81·9	75·9
Sept. "	467	170	36·3	82·2	75·5
Oct. "	443	161	36·5	71·8	77·0
Nov. "	325	128	39·5	55·6	74·9
Dec. "	327	128	38·7	62·3	70·8
Jan. 1910	346	93	26·4	52·1	69·7
Feb. "	384	38	10·6	39·3	72·0
March "	340	6	1·7	37·9	78·9
April "	161	nil	—	30·3	84·5
May "	179	20	10·2	47·6	85·1
June "	482	175	37·3	73·2	78·8
July "	621	268	43·2	77·1	77·4
Aug. "	731	238	32·6	86·8	75·4
Sept. "	577	220	38·1	84·1	75·8
Oct. "	609	256	42·0	76·2	75·7
Nov. "	467	185	39·6	73·7	68·6
Dec. "	624	268	42·9	67·1	66·8
Jan. 1911	497	177	35·6	64·9	70·9
Feb. "	390	70	17·9	47·7	71·7
March "	253	48	18·9	52·5	76·8
April "	178	6	3·4	43·7	83·9
May "	123	7	5·7	52·0	87·3
June "	271	115	42·4	77·2	79·9
July "	387	138	35·7	75·6	78·4
Aug. "	364	98	26·9	85·5	74·9
Sept. "	424	175	41·3	79·9	76·4
Oct. "	309	98	31·7	75·4	81·0
Nov. "	264	78	29·5	66·9	76·4
Dec. "	242	29	12·0	62·9	71·6
Jan. 1912	177	31	17·5	57·7	71·3
Feb. "	137	22	16·1	64·0	74·3

In table V the proportion of eggs hatching on successive days is shown, the period of incubation being longer during the cooler months.

In order to ascertain the relative importance of temperature and humidity on the rate of egg hatching, the following experiments were also carried out.

TABLE V.

*Table showing the percentage of eggs hatched to the total number hatched on successive days from the date on which they were laid.*

Month	Average 8 o'clock humidity	Average mean temperature	Percentage of flea-eggs hatched	Percentage of flea-eggs hatched to the total hatched on successive days from date of keeping them												
				4	5	6	7	8	9	10	11	12	13	14	15	16
July 1909	84.4	75.3	21.8	—	32.5	36.1	26.5	3.6	1.2	—	—	—	—	—	—	—
Aug. "	81.9	75.9	31.9	—	49.7	44.1	4.8	0.7	0.7	—	—	—	—	—	—	—
Sept. "	82.2	75.5	36.4	8.8	25.9	51.8	10.0	2.9	0.6	—	—	—	—	—	—	—
Oct. "	71.8	77.0	36.3	2.5	54.7	39.8	2.5	0.6	—	—	—	—	—	—	—	—
Nov. "	55.6	74.9	39.1	—	6.2	28.9	35.2	22.7	5.5	1.6	—	—	—	—	—	—
Dec. "	62.3	70.8	39.1	—	—	30.5	60.2	6.2	2.5	0.8	—	—	—	—	—	—
Jan. 1910	52.1	69.7	26.9	—	—	—	40.9	30.1	19.4	9.7	—	—	—	—	—	—
Feb. "	39.3	72.0	9.9	—	—	10.5	68.4	7.9	5.3	5.3	—	3.6	—	—	—	—
March "	37.9	78.9	1.8	—	—	—	50.0	50.0	—	—	—	—	—	—	—	—
April "	30.3	84.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
May "	47.6	85.1	11.2	—	85.0	15.0	—	—	—	—	—	—	—	—	—	—
June "	73.2	70.8	36.3	11.4	45.1	34.9	5.7	2.3	0.6	—	—	—	—	—	—	—
July "	77.1	77.4	43.2	2.2	66.8	24.3	5.2	0.7	0.4	0.4	—	—	—	—	—	—
August "	86.8	75.4	32.6	—	35.7	42.0	17.2	4.2	0.8	—	—	—	—	—	—	—
Sept. "	84.1	75.8	38.1	—	46.4	35.0	12.3	0.5	0.9	0.5	—	—	—	—	—	—
Oct. "	76.2	75.7	42.0	—	36.7	53.1	9.4	—	0.4	—	—	—	—	—	—	—
Nov. "	73.7	68.6	39.6	0.5	0.5	25.0	10.0	8.7	14.6	14.6	11.9	—	1.1	0.5	1.5	1.1
Dec. "	67.1	66.8	42.9	—	—	—	3.4	39.1	41.4	13.4	1.5	1.1	—	—	—	—
Jan. 1911	64.9	70.9	35.6	—	—	19.2	45.8	19.8	5.6	9.0	0.6	—	—	—	—	—
Feb. "	47.7	71.7	17.9	—	—	15.7	35.7	21.4	5.7	7.1	7.1	—	7.1	—	—	—
March "	52.5	76.8	18.9	—	12.5	60.4	25.0	—	2.1	—	—	—	—	—	—	—
April "	43.7	83.9	3.4	—	66.6	33.3	—	—	—	—	—	—	—	—	—	—
May "	52.0	87.3	5.7	28.6	28.6	42.8	—	—	—	—	—	—	—	—	—	—
June "	77.2	79.9	42.4	11.4	71.3	13.0	1.7	2.6	—	—	—	—	—	—	—	—

(b) *Influence of a varying humidity on egg hatching.*

Test tubes containing eggs were divided into three batches, and after being plugged with cotton wool were capped with india-rubber. In one batch the plug of cotton wool was moistened with a drop of water. In another one eighth of a Kodak film drying disc was placed, and the third was kept under the ordinary atmospheric conditions prevailing at the time. The results of these observations are seen in table VI

TABLE VI.

*Table comparing the percentage of flea-eggs hatched into larvae at room temperature in test tubes having different degrees of humidity.*

Month	Average 8 o'clock humidity	Average mean temperature	A. Normal			B. Moist			C. Dry		
			No. of eggs kept	No. of larvae found	Percentage	No. of eggs kept	No. of larvae found	Percentage	No. of eggs kept	No. of larvae found	Percentage
Jan. 1911...	64.9	70.9	52	17	32.7	53	26	49.1	19	—	nil
Feb. „ ...	47.7	71.7	57	15	26.3	29	12	41.4	41	—	nil
March „ ...	52.5	76.8	43	10	23.3	41	16	39.0	—	—	—
April „ ...	43.7	83.9	24	2	8.3	28	12	42.9	6	—	nil
May „ ...	52.0	87.3	54	2	3.7	50	1	2.0	—	—	—
June „ ...	77.2	79.9	156	69	44.2	39	7	17.9	23	2	8.7
July „ ...	75.6	78.4	118	45	38.1	109	39	35.8	104	6	5.8
Aug. „ ...	85.5	74.9	133	38	28.6	40	8	20.0	29	6	20.7
Sept. „ ...	79.9	76.4	90	27	30.0	32	11	34.4	20	—	—
Totals	...	...	727	225	30.9	421	132	31.4	242	14	5.8
Average monthly percentage			—	—	26.1	—	—	31.4	—	—	3.9

A. Normal amount of humidity for the period (tube capped).

B. One drop of distilled water put on to the plug (tube capped).

C. One-eighth of a drying disc obtained from a roll of Kodak films placed inside the cotton plug (tube capped).

which shows that, compared with the tube kept under room conditions, the artificial raising of the humidity was distinctly favourable during the naturally dry months; but the difference was not so marked, indeed it was occasionally less favourable, in the months of the rainy season, especially in June and August. Artificial lowering of the humidity on the other hand was distinctly unfavourable in the dry season, when the atmospheric humidity was already very low. In the rainy season with this lowered humidity there was still a marked difference, except in the month of August when sufficient humidity

was apparently not removed to interfere with the process of egg hatching. It was noticed that when the humidity was exceptionally high, moulds formed readily in the tubes, especially on the eggs, from which their mycelia could be seen radiating in every direction, and it is possible that these may have killed the eggs. It will be noticed

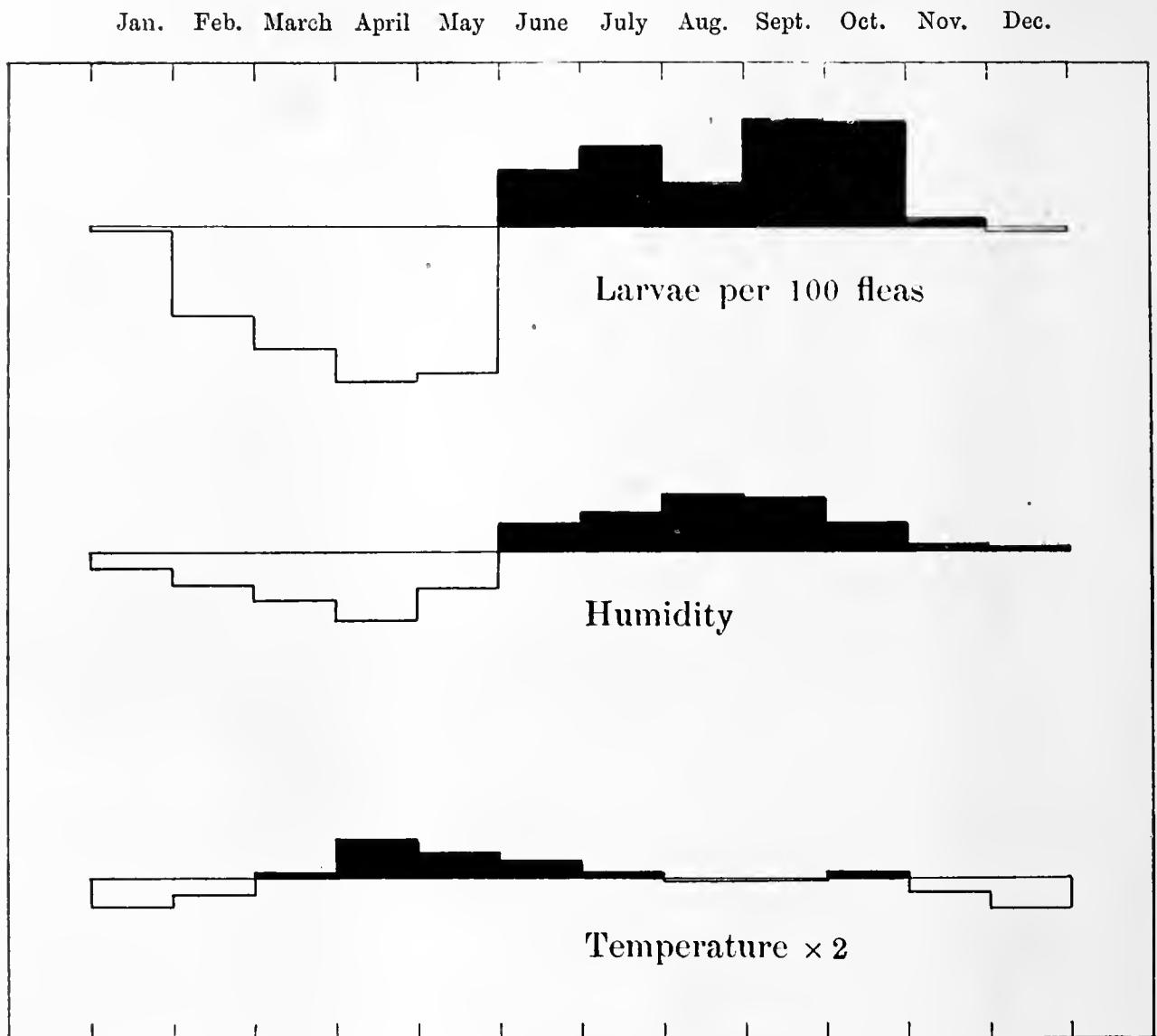
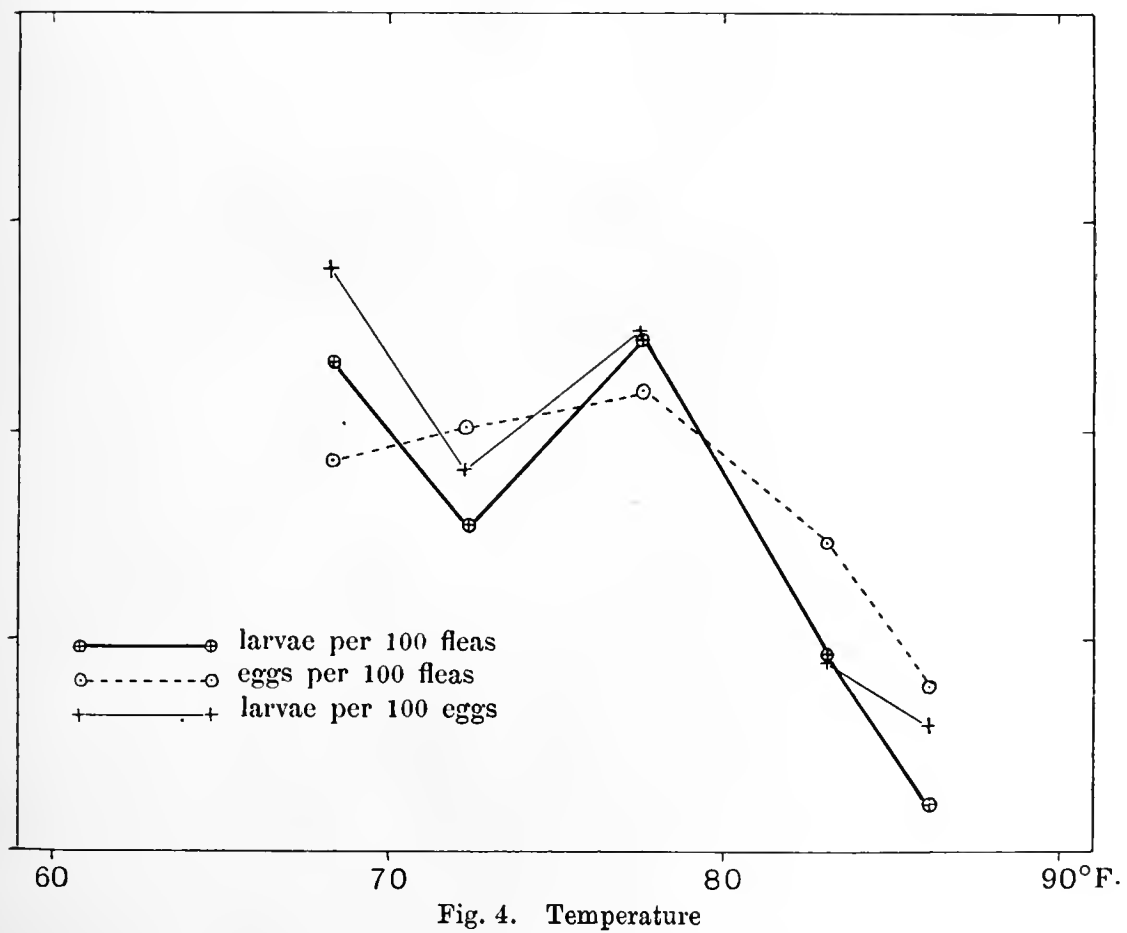
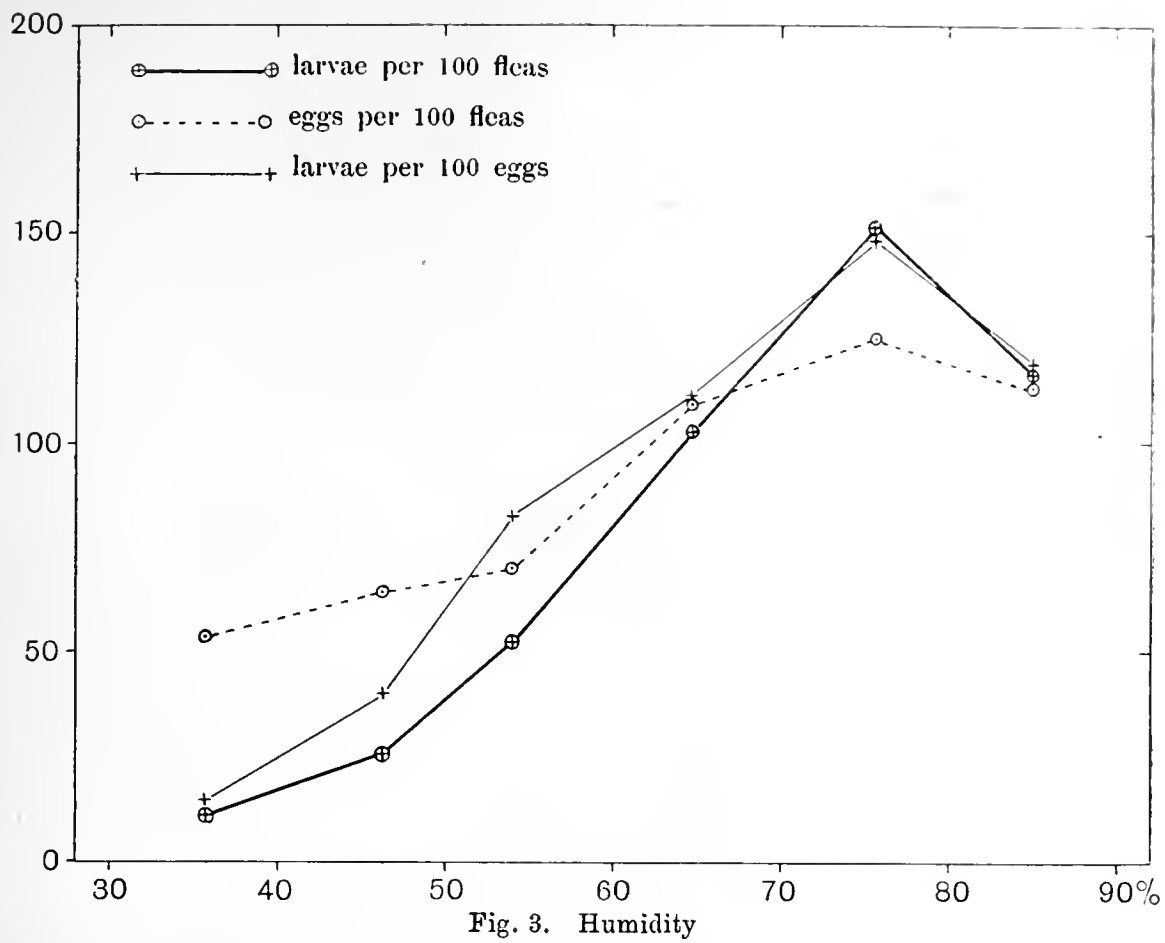


Fig. 2.

that in the month of August when the average humidity (85.5%) was considerably higher than in any of the other months, fewer eggs were hatched under ordinary atmospheric conditions than there were during June, July and September. Moreover by further raising the humidity during this month the number of eggs hatched fell still further, that is, from 28.6% to 20.0%. The latter figure was even rather less than



that obtained in the "dry tube" which gave 20·7 % hatched. It would appear therefore that at the temperature then prevailing, *i.e.* about 74·9° F., an average atmospheric humidity above 85 is already detrimental to the hatching of fleas' eggs. When this humidity is further raised it becomes distinctly more unfavourable. It is probable that, in this month, even under ordinary atmospheric conditions the formation of moulds, as we have already explained, may have interfered considerably with the hatching of eggs, but whether this occurs also in nature we cannot tell.

(c) *Influence of varying temperature on egg hatching.*

A variation in the temperature naturally affects the percentage of atmospheric humidity; it was therefore difficult to keep the latter at a constant figure over a period such as is required for the hatching of eggs, and the further development of these through the various stages in the life history of the flea. An attempt was made to eliminate the humidity factor by drying the air in each test tube by means of drying discs, but in this case none of the 61 eggs kept at a higher temperature, nor 51 kept at the room temperature, nor 36 kept at a low temperature hatched into larvae.

(d) The number of eggs laid per 100 fleas (in an hour) being recorded for each month during our observations as well as the number of these that hatched into larvae, the number of larvae obtained from the eggs laid by every 100 fleas kept for one hour could be ascertained. This recorded for each month is shown in table VII, together with the egg laying and hatching figures separately. In order that the three series may be readily compared, the data have been also calculated relative to the average figures for the complete double cycle from January 1910 to December 1911 taken as 100. These summary figures show very plainly that the seasonal changes in egg laying and in egg hatching run together with the result that more than twenty times as many larvae were obtained in September and October, when it was warm and wet, as in April and May, when it was hot and dry.

In Poona the mean temperature varies comparatively little at different seasons of the year, and much less than the humidity. The hottest months are also the driest though in the cooler season the humidity is not at its maximum. It is a matter of some interest to disentangle as far as possible the influence of humidity from that of

TABLE VII.

*Table showing the number of larvae obtained from the eggs laid in one hour by a hundred fleas in monthly periods.*

Month		Eggs per 100 fleas		Percentage of eggs hatched		Larvae per 100 fleas	
		Absolute	Relative*	Absolute	Relative*	Absolute	Relative*
July	1909	159	80	20·9	80	33	55
August	"	235	<b>119</b>	31·9	<b>122</b>	75	<b>125</b>
September	"	208	<b>105</b>	36·3	<b>139</b>	75	<b>125</b>
October	"	199	<b>100</b>	36·5	<b>139</b>	73	<b>122</b>
November	"	133	67	39·5	<b>151</b>	53	88
December	"	135	68	38·7	<b>147</b>	52	87
January	1910	132	67	26·4	<b>101</b>	35	58
February	"	169	85	10·6	40	18	30
March	"	84	42	1·7	6	1	2
April	"	62	31	0·0	0	0	0
May	"	84	42	10·2	39	9	15
June	"	167	84	37·3	<b>143</b>	62	<b>103</b>
July	"	260	<b>131</b>	43·2	<b>165</b>	112	<b>120</b>
August	"	276	<b>140</b>	32·6	<b>125</b>	90	<b>150</b>
September	"	223	<b>113</b>	38·1	<b>145</b>	84	<b>140</b>
October	"	296	<b>150</b>	42·0	<b>161</b>	124	<b>207</b>
November	"	199	<b>100</b>	39·6	<b>151</b>	79	<b>132</b>
December	"	220	<b>111</b>	42·9	<b>164</b>	94	<b>157</b>
January	1911	295	<b>149</b>	35·6	<b>136</b>	105	<b>175</b>
February	"	185	93	17·9	68	33	55
March	"	150	76	18·9	72	28	47
April	"	115	58	3·4	13	4	7
May	"	72	36	5·7	22	4	7
June	"	230	<b>116</b>	42·4	<b>162</b>	98	<b>163</b>
July	"	304	<b>153</b>	35·7	<b>136</b>	109	<b>182</b>
August	"	242	<b>122</b>	26·9	<b>103</b>	65	<b>108</b>
September	"	279	<b>141</b>	41·3	<b>158</b>	115	<b>192</b>
October	"	257	<b>130</b>	31·7	<b>121</b>	81	<b>135</b>
November	"	204	<b>103</b>	29·5	<b>113</b>	60	<b>100</b>
December	"	242	<b>122</b>	12·0	46	29	48
January	1912	206	<b>104</b>	17·5	67	36	60
February	"	202	<b>102</b>	16·1	61	32	53
Average Jan. 1910 to Dec. 1911		198	100	26·2	100	60	100

\* Relative to the mean values from January 1910 to December 1911.

temperature. To some extent this may be done with the figures given in table VII. If these are grouped according to humidities (table VIII), there is a regular increase in larva production up to 80% saturation which shows no particular relation to the average temperature of each group of months. The fall in the most humid group, with a medium temperature, is perhaps associated with the excessive growth of moulds

under these conditions which has been already mentioned. On the other hand, the same figures grouped according to the dry bulb thermometer (table IX) show far less regularity. On the whole therefore it appears

TABLE VIII.

*Mean relative figures of table VII grouped by humidities.*

	Group	No. of months	Mean humidity	Mean temperature	Eggs per 100 fleas	Percent. hatched	Larvae per 100 fleas
I	30—40 %	3	35.8	78.5	53	15	11
II	40—50 %	3	46.3	80.2	64	40	26
III	50—60 %	5	54.0	76.0	70	83	52
IV	60—70 %	6	64.7	71.6	109	111	103
V	70—80 %	9	75.6	77.1	123	148	151
VI	80—90 %	6	85.0	75.5	113	119	117

TABLE IX.

*Mean relative figures of table VII grouped by temperatures.*

	Group	No. of months	Mean humidity	Mean temperature	Eggs per 100 fleas	Percent. hatched	Larvae per 100 fleas
I	65—70°	3	64.3	68.4	93	139	116
II	70—75°	9	60.0	72.4	101	91	78
III	75—80°	15	74.2	77.6	110	124	122
IV	80—85°	3	49.8	83.1	73	45	47
V	85—90°	2	49.8	86.2	39	30	11

TABLE X.

	Month	Humidity	Temperature	Eggs per 100 fleas	Percent. hatched	Larvae per 100 fleas
	March 1910	37.9	78.9	42	6	2
	„ 1911	52.5	76.8	76	72	47
	Nov. „	66.9	76.4	103	113	100
	Oct. 1909	71.8	77.0	100	139	122
	June 1910	73.2	79.8	84	143	103
	July 1911	75.6	78.4	153	136	182
	Oct. 1910	76.2	75.7	150	161	207
	July „	77.1	77.4	131	165	120
	June 1911	77.2	79.9	116	162	163
	Sept. „	79.9	76.4	141	158	192
	Aug. 1909	81.0	75.9	119	122	125
	Sept. 1910	84.1	75.8	113	145	140
	July 1909	84.4	75.3	80	80	55
	Aug. 1910	86.8	75.4	140	125	150
	Sept. 1909	88.2	75.5	105	139	125

likely that, with the range of temperature obtaining in Poona, the seasonal variation in larva production in these experiments was conditioned more by the favouring influence of a high humidity than by the destructive effect of an excessive temperature. Much the same is indicated by the figures for the individual months in any one group.



Table X gives the figures for the fifteen months in which the temperature fell between 75·0° and 79·9° while the humidity ranged from 38 % to 88 %.

Table VII also appears to indicate that there is a greater seasonal variation in the percentage of eggs hatching into larvae than in the number of eggs laid. Since both these stages have a seasonal variation in the same sense, the number of larvae obtained per flea, which is the product of them both, naturally shows the widest range<sup>1</sup>. In the artificially humidified experiments there is the same difference between normal and moist conditions in egg laying (table II) as in egg hatching (table VI).

### III. THE DEVELOPMENT OF THE LARVAE INTO PUPAE AND INTO FULLY FORMED FLEAS.

#### (a) *Seasonal variation.*

These observations were commenced in August 1910. The larvae when hatched out of eggs were kept in test tubes and fed on wheat bran. Since the middle of July 1911 an attempt was made to sterilize

TABLE XI.

*Table showing the number and percentage of fleas formed from the larvae of Xenopsylla cheopis allowed to feed on wheat bran at room temperature and at normal atmospheric humidity.*

Month	Average mean temperature	Average 8 o'clock humidity	No. of larvae kept	No. of fleas formed	Percentage of fleas formed
Aug. 1910	75·4	86·8	133	16	12·0
Sept. „	75·8	84·1	167	8	4·8
Oct. „	75·7	76·2	107	0	nil
Nov. „	68·6	73·7	45	0	nil
Dec. „	66·8	67·1	79	0	nil
Jan. 1911	70·9	64·9	152	0	nil
Feb. „	71·7	47·7	72	0	nil
March „	76·8	52·5	44	0	nil
April „	83·9	43·7	7	0	nil
May „	87·3	52·0	No larvae kept		
June „	79·9	77·2	40	9	22·5
July „	78·4	75·6	94	19	20·2
Aug. „	74·9	85·5	56	7	12·5
Sept. „	76·4	79·9	88	5	5·7
Oct. „	81·0	75·4	59	0	nil
Nov. „	76·4	66·9	22	0	nil
Dec. „	71·6	62·9	32	0	nil

<sup>1</sup> The method is not altogether valid in the present instance, but we may note that the variabilities (coefficient of variation) of the figures in table VII are 34, 50 and 63 for egg laying, egg hatching and larvae respectively.

the bran by heating. The movements of the larvae and the formation of cocoons were noted from time to time, and the further development of the young fleas was watched. The results are shown in table XI. It will be seen that the greatest percentage of fleas were developed from larvae during June and July, fewer were found in August and very few in September, whilst none were found during the remaining months of the year. No larvae were kept for observation during May as none were available<sup>1</sup>.

(b) *The influence of varying humidity on the development of larvae.*

The larvae were kept in two batches in test tubes plugged with cotton wool and capped with a rubber cap. Small pieces of blotting paper 2 mm. square were put into each: in the one case they were dry, and in the other they were moistened with water. The larvae were then kept under observation in a dark box as already described. The result will be seen in table XII. It will be noticed that in the normal atmosphere cocoons were visible only during the months of June to October inclusive, and fleas were found only from June to September inclusive. It will be seen that the moist atmosphere however was considerably more favourable to the life of the larvae and their development into pupae and fleas than a dry atmosphere. It is of interest to note that during the humid months of August and September—which under normal conditions are favourable to the development of pupae and larvae—when the humidity was still further raised, it appears to be detrimental to their development, for comparatively few fleas were found. This is probably due to the growth of the moulds, for the bran was on several occasions seen to be mouldy during these months. These figures must be taken with some caution for it must be remembered that, as we have already mentioned, our results in this experiment were at certain seasons unfortunately based on very small figures.

<sup>1</sup> It is to be regretted that during the drier months of the year these results are based on very small figures. This was due to the fact that the number of fleas during these months was very limited, and moreover as we have seen very few eggs also were laid, and scarcely any of these were hatched in the course of our experiments.

TABLE XII.

Table comparing the development of larvae (*Xenopsylla cheopis*) into cocoons and fleas when at room temperature and at different degrees of humidity.

Month, 1911	A. Normal atmospheric condition										B. Moist			
	Average humidity at 6 o'clock	Number of larvae kept	Mean day till which larvae were active	Cocoons visible		Fleas formed		Number of larvae kept	Mean day till which larvae were active	Number formed	Cocoons visible		Fleas formed	
				Number formed	Percentage of larvae kept	Number formed	Percentage of larvae kept				Number formed	Percentage of larvae kept	Number formed	Percentage of larvae kept
January	64.9	94	18.2	—	—	—	—	95	24.2	4	4	4.2	4	4.2
February	47.7	78	7.5	—	—	—	—	76	20.2	6	6	7.9	9	11.8
March	52.5	44	7.0	—	—	—	—	42	24.5	3	3	7.1	6	14.3
April	43.7	7	10.0	—	—	—	—	5	10.5	—	—	—	—	—
May	52.0	—	—	—	—	—	—	—	—	—	—	—	—	—
June	77.2	40	28.0	1	2.5	—	—	38†	25.7	2	2	5.3	12	31.6
July*	75.6	94	31.6	13	13.8	19	20.2	91†	24.4	10	10	11.0	12	13.2
August	85.5	56	32.7	7	12.5	7	12.5	56§	28.5	—	—	—	1	1.8
September	79.9	88	40.7	5	5.7	5	5.7	54	48.0	2	2	3.7	1	1.9
October	75.4	59	10.0	1	1.7	—	—	59	31.5	5	5	8.5	1	1.7
November	66.9	22	10.7	—	—	—	—	21	24.7	1	1	4.8	—	—
December	62.9	32	11.7	—	—	—	—	31	25.5	—	—	—	—	—
		614	19.0	27	4.4	40	6.5	568	26.2	33	33	5.8	46	8.1

A. A capped glass test tube representing normal amount of humidity for the period. A 2 millimetre square piece of ordinary blotting paper put inside bran as a control.

B. A capped test tube having humidity artificially raised above the normal by putting a 2 mm. square piece of moistened blotting paper inside the bran.

\* Since the middle of this month, the bran in which the larvae were fed was sterilized by dry heat.

† The bran in which 23 of these larvae were fed became mouldy.

‡ " " " " " "

§ " " " " " "

TABLE XIII.

*Table showing the average life in days of a rat flea when kept isolated in glass test tubes without feeding.*

Half-month ending	Number of fleas observed	Number of days lived	Average life in days	Average 8 o'clock humidity
15 July 1910	65	194.0	2.98	77.4
31 „ „	55	158.0	2.88	76.8
15 August „	90	262.5	2.92	87.5
31 „ „	65	197.5	3.04	86.0
15 September „	50	173.5	3.46	82.1
30 „ „	25	61.0	2.44	86.0
15 October „	60	150.0	2.50	79.9
31 „ „	70	224.5	3.20	72.5
15 November „	45	127.0	2.82	77.4
30 „ „	45	114.5	2.54	69.9
15 December „	60	162.5	2.70	64.2
31 „ „	60	188.5	3.14	70.0
15 January 1911	45	147.0	3.26	67.1
31 „ „	40	111.0	2.78	62.6
15 February „	50	102.5	2.04	45.5
28 „ „	70	98.0	1.40	49.9
15 March „	45	72.5	1.62	52.5
31 „ „	65	70.0	1.08	46.5
15 April „	65	59.5	0.92	44.8
30 „ „	55	40.5	0.74	42.5
15 May „	45	44.0	0.98	45.9
31 „ „	45	61.5	1.36	52.1
15 June „	45	135.5	3.02	80.2
30 „ „	40	165.0	4.12	74.3
15 July „	55	172.5	3.14	77.4
31 „ „	50	134.0	2.68	73.8
15 August „	55	256.0	4.66	84.7
31 „ „	45	196.0	4.36	86.4
15 September „	35	120.5	3.44	83.3
30 „ „	35	126.0	3.60	76.5
15 October „	40	116.0	2.92	79.0
31 „ „	45	101.0	2.24	71.9
15 November „	45	75.5	1.68	58.6
30 „ „	40	160.5	4.02	75.3
15 December „	35	132.0	3.78	68.0
31 „ „	60	182.0	3.04	57.9
15 January 1912	40	82.5	2.06	59.4
31 „ „	35	70.5	2.02	56.0
15 February „	25	60.5	2.42	74.5
28 „ „	25	50.5	2.02	64.0
15 March „	20	17.0	0.85	56.6

IV. THE LENGTH OF LIFE OF A FLEA<sup>1</sup>.

(a) *Seasonal variations.*

If fleas conveyed in luggage or merchandise (such as grain) may be the means of transmitting plague from one place to another over considerable distances, the length of the life of a flea whilst so confined (*i.e.* without feeding), if found to vary much at different seasons, might be a factor of considerable importance, favourable or otherwise, to the spread of plague from village to village or from one district to another. On the facility with which plague infection can be so transported, the severity of an epidemic in a district would also largely depend.

With a view to determining what seasonal variations, if any, there are in the length of life of a flea the following observations were undertaken. Fully grown fleas were selected and kept in capped glass test tubes together with small pieces of cloth. The number dying on successive days was noted. The average length of life of a flea was then calculated in periods of half a month.

The results are shown in table XIII compared with the half-monthly average 8 a.m. humidity<sup>2</sup>. It will be seen that there is a marked correlation between the length of their life and the humidity. The longest life is during August when the humidity is over 80% and their shortest life in April and the first half of May, when the humidity is about 45% or less. It is of interest to note that in August they were, under the conditions observed in the experiment, found to live

TABLE XIV.

*Table showing the average number of days which one rat flea lived when kept isolated in glass test tubes without feeding.*

Half-month ending	Number of fleas kept	Total life	Average	Average 8 o'clock humidity (half-monthly)	Average half-monthly mean temperature
March 15, 1911	15	27·5	1·8	58·5	74·6
„ 31 „	10	2·0	0·2	46·5	79·0
April 15 „	25	22·5	0·9	44·8	83·1
„ 30 „	20	12·0	0·6	42·5	84·7
May 15 „	25	10·0	0·4	45·9	87·6
„ 31 „	50	37·5	0·7	58·1	87·0
June 15 „	30	75·5	2·5	80·2	80·7
„ 30 „	25	50·5	2·0	74·3	79·1

<sup>1</sup> For observations on *Ceratophyllus fasciatus* see W. Nicoll, *Brit. Med. Journ.* 1912, vol. II. p. 926.

<sup>2</sup> The monthly temperatures and humidities are given in table I above.

about five times longer than they do in April, in other words, plague-infected fleas could under similar conditions be conveyed five times farther in August than they could be in April. Table XIV similarly gives the length of life of unselected fleas. It will be seen that a similar variation takes place if full-sized fleas are not selected.

(b) *Influence of varying humidity.*

In a manner similar to the previous experiments already described, fleas were put into test tubes and divided into three batches. In one the humidity was artificially raised by means of a drop of water, in another it was lowered by means of a piece of a photographic drying disc, the third batch being kept capped under the atmospheric conditions of the room. The result is found in table XV; it will be seen that, in almost every month, the effect of the addition of moisture prolonged the life of the flea, the contrast was most marked in the months of the hot dry weather. The abstraction of moisture, on the other hand, almost

TABLE XV.

*Table comparing the average life in days of X. cheopis kept in the dark at room temperature in glass test tubes having different degrees of humidity.*

Month		Average 8 o'clock humidity	Average mean temperature	A			B			C		
				Normal			Moist			Dry		
				Fleas kept	Total life in days	Average life of one flea in days	Fleas kept	Total life in days	Average life of one flea in days	Fleas kept	Total life in days	Average life of one flea in days
Jan. 1911		64.9	70.9	15	55.0	3.7	15	61.5	4.1	10	13.5	1.3
Feb. „		47.7	71.7	10	17.5	1.7	10	44.0	4.4	5	11.0	2.2
March „		52.5	76.8	10	6.0	0.6	15	55.0	3.7	15	6.0	0.4
April „		43.7	83.9	10	4.5	0.4	10	19.5	1.9	—	—	—
May „		52.0	87.3	No observations made								
June „		77.3	79.9									
July „		75.6	78.4	15	43.5	2.9	10	29.5	2.9	10	10.0	1.0
Aug. „		85.5	74.9	45	192.5	4.3	35	254.5	7.3	30	59.0	1.9
Sept. „		79.9	76.4	20	70.0	3.5	10	58.0	5.8	10	13.0	1.3
Oct. „		75.4	81.0	15	45.0	3.0	15	57.5	3.8	10	7.0	0.7
Totals		—	—	140	434.0	3.1	120	579.5	4.8	90	119.5	1.3

A. Without any moisture or drying disc (tube capped).

B. One drop of distilled water added on to the cotton plug (tube capped).

C. One-eighth of a drying disc obtained from a roll of Kodak films, placed inside the cotton plug (tube capped).

always shortened the length of the life of a flea. The only exception occurred in the month of February but this may be accounted for by the fact that the figures were too small to strike an average, as in this month observations were made on only five fleas kept in a dry atmosphere.

(c) *Influence of varying temperature.*

It was somewhat difficult to keep the humidity constant under varying conditions of temperature for a period extending over several days, so in a manner similar to the experiment (p. 302) already described, an attempt was made to eliminate the effect of moisture by means of a small piece of photographic drying disc kept in the tubes. The tubes containing fleas were then kept under varying conditions of temperature, and the length of life of the flea was observed in the usual manner.

TABLE XVI.

*Table showing the average life of one full grown cheopis kept without feeding at different temperatures, the atmosphere of the tubes having been rendered dry by a piece of drying disc placed inside them prior to capping.*

Date	A Incubator 98°—104° F. = 101° F.		B Room 55°—85° F. = 70° F.		C Ice-box 55°—65° F. = 60° F.	
	Fleas kept	Average life of one in days	Fleas kept	Average life of one in days	Fleas kept	Average life of one in days
December 15, 1911	5	0·0	5	1·5	5	2·2
„ 16 „	5	0·5	5	0·5	5	1·1
„ 18 „	5	0·0	5	1·0	5	1·4
„ 20 „	5	0·0	5	0·2	5	0·2
„ 21 „	5	0·0	5	1·4	5	1·2
„ 22 „	5	0·0	5	2·4	5	2·2
„ 26 „	5	0·0	5	1·0	5	1·6
„ 29 „	5	0·3	5	1·5	5	2·1
„ 30 „	5	0·5	5	1·0	5	1·6
January 4, 1912	5	0·0	5	1·3	5	2·2
„ 6 „	5	0·5	5	1·1	5	2·2
„ 23 „	5	0·0	0	0	5	1·2
February 2 „	5	0·0	0	0	5	2·4
Total fleas kept and the mean average life of one flea	65	0·14	55	1·17	65	1·66

The result is seen in table XVI. It will be seen that the average length of life of the flea was considerably longer in the low temperature

(55°—65° F.) than in the room temperature (55°—85° F.); in this again it was considerably longer than in the warm temperature 98°—104° F.

An analysis of the monthly figures for 20 months given in table XIII with reference to humidity and temperature is shown in tables XVII and XVIII and graphically in figures 5 and 6. The relation to humidity appears to be more regular and definite than to temperature.

TABLE XVII.

*Average monthly figures of table XIII grouped by humidities.*

Group	No. of months	Mean humidity	Mean temperature	Average duration of life
I 30—40 %	0	—	—	—
II 40—50 %	2	45.7 %	77.8°	1.27
III 50—60 %	3	54.1	78.5	1.52
IV 60—70 %	5	65.1	71.8	2.88
V 70—80 %	7	76.4	76.8	3.01
VI 80—90 %	3	85.5	75.4	3.48

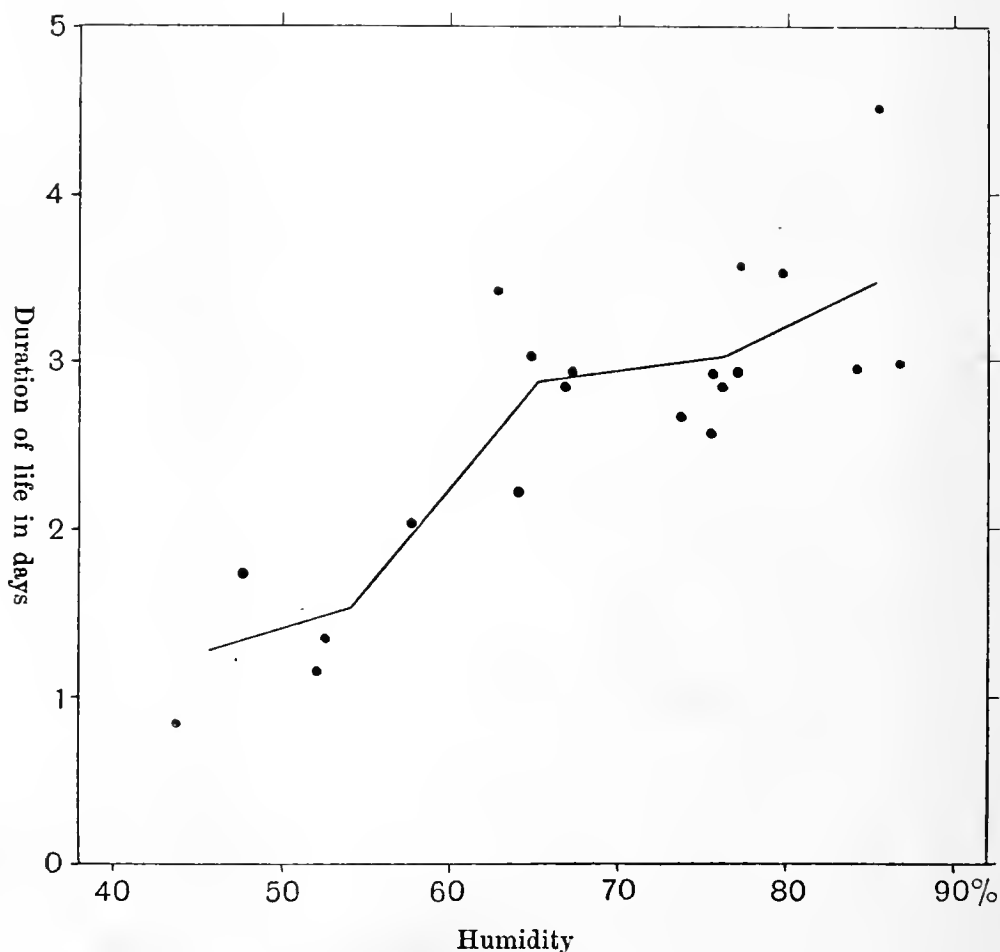


Fig. 5.



TABLE XVIII.

*Average monthly figures of table XIII grouped by temperatures.*

Group	No. of months	Mean humidity	Mean temperature	Average duration of life
I 65—70°	2	70·4 %	67·7°	2·80
II 70—75°	6	63·8	72·3	2·82
III 75—80°	9	75·1	76·9	2·88
IV 80—85°	2	60·0	82·5	1·70
V 85—90°	1	52·0	87·3	1·35

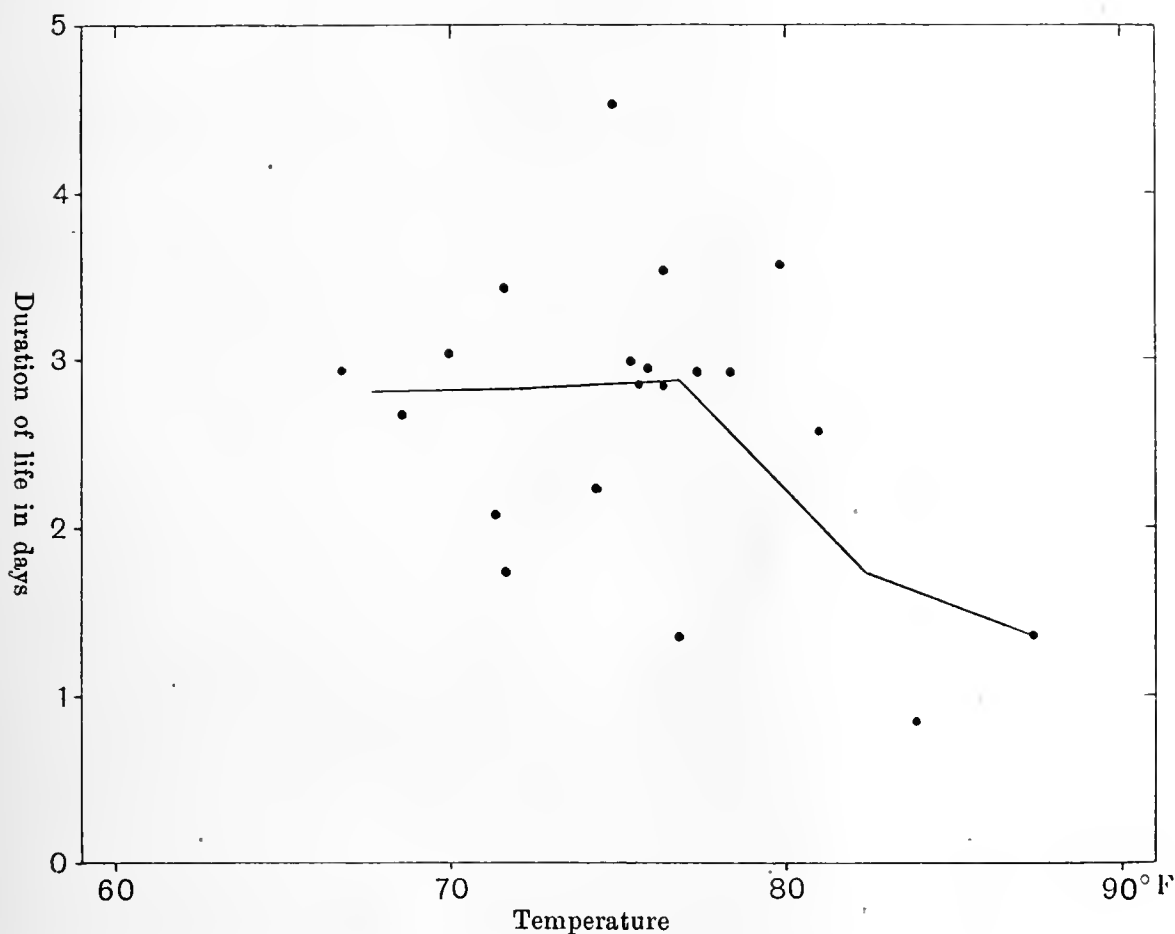


Fig. 6.

(d) *The length of life of a newly-hatched flea.*

Newly-hatched and fully-developed fleas were kept in test tubes without feeding and the length of their lives noted. The result will be seen in table XIX to be that, under the conditions of the experiment, the length of life of a newly-hatched flea that has never fed is considerably longer than that of an adult.

TABLE XIX.

Table comparing the average life of a fully-grown flea with that of a newly-hatched flea under similar conditions.

Half-month ending	Fully-grown flea			Newly-hatched flea		
	Fleas kept	Total life in days	Average	Fleas kept	Total life in days	Average
28 February, 1911	70	98·0	1·4	1	5·0	5·0
15 March „	45	72·5	1·6	3	12·5	4·2
31 „ „	65	70·0	1·1	1	7·0	7·0
15 May „	45	44·0	1·0	1	2·0	2·0
15 July „	55	172·5	3·1	9	81·5	9·1
31 „ „	50	134·0	2·7	13	97·0	7·5
15 August „	60	282·0	4·7	9	75·0	8·3
31 „ „	45	196·0	4·4	12	116·0	9·7
15 September „	35	120·5	3·4	13	57·5	4·4
30 „ „	35	126·0	3·6	1	3·0	3·0
15 October „	40	116·5	2·9	1	4·0	4·0
Total	545	1432	2·6	64	460·5	7·2

V. RELATION TO NATURAL PREVALENCE OF FLEAS.

Observations on the natural prevalence of fleas on wild *M. rattus* in Poona were made by the usual method continuously from July 1908 to February 1912. Some of the figures have been already published

TABLE XX.

Month	Fleas per 100 rats		Relative figures for				Humidity	Tempera- ture	Average relative weekly plague mortality†
	Absolute	Relative *	Egg laying	Egg hatching	Larvae	Length of life			
Jan.	433	90	<b>107</b>	<b>101</b>	98	<b>105</b>	58·2 %	70·3°	<b>156</b>
Feb.	437	91	93	57	46	82	50·3	72·7	<b>103</b>
March	397	83	59	39	25	49	45·2	77·8	35
April	290	61	44	6	3	34	37·0	84·2	12
May	213	45	39	30	10	48	49·8	81·2	7
June	240	50	<b>100</b>	<b>152</b>	<b>133</b>	<b>148</b>	75·2	79·8	8
July	520	<b>109</b>	<b>122</b>	<b>127</b>	<b>141</b>	<b>121</b>	79·0	77·0	45
August	825	<b>173</b>	<b>127</b>	<b>116</b>	<b>128</b>	<b>155</b>	84·4	75·4	<b>140</b>
Sept.	880	<b>184</b>	<b>119</b>	<b>147</b>	<b>153</b>	<b>134</b>	84·1	75·9	<b>200</b>
October	765	<b>160</b>	<b>127</b>	<b>140</b>	<b>155</b>	<b>112</b>	74·5	77·9	<b>203</b>
Nov.	540	<b>113</b>	91	<b>138</b>	<b>106</b>	<b>114</b>	65·4	73·3	<b>151</b>
Dec.	480	<b>100</b>	<b>100</b>	<b>119</b>	98	<b>131</b>	64·1	69·7	<b>139</b>

\* To the average monthly flea prevalence (479) for the 24 months, January 1909 to December 1910.

† See Vol. x. p. 495.

(Vol. x. p. 525), and the whole series will be described in detail in a subsequent report. Neglecting for the moment the interannual variations, we give in table XX the average monthly figures for the whole period, comparing them with the data obtained by artificial breeding.

There is thus a good correspondence between the results of artificial breeding and the prevalence of fleas in nature. The marked increase in egg laying and hatching occurs in June with the onset of wet weather and the prevalence of imagines in nature follows in July.

## VI. SUMMARY.

1. *The laying of eggs by wild fleas, the development of the eggs into larvae and the development of larvae into pupae and imagines all show a marked seasonal variation, being most active when the weather is wet and the temperature moderate and least active under dry and hot conditions.*

2. *Within the range of temperatures obtaining in Poona, atmospheric humidity seems to be a more important factor than temperature in conditioning this seasonal variation.*

3. *Artificial humidification of the atmosphere is favourable to all stages of the metamorphosis especially in the dry season.*

4. *The seasonal variations in the results of artificial breeding correspond in time to the seasonal variations in the prevalence of fleas on rats in nature.*

5. *The life of an adult flea is longer in a cool and moist atmosphere than under hot and dry conditions.*

Owing to the failure to breed out imagines except in the most favourable months, it is not possible to give any numerical expression to the seasonal variation in the abundance of the second generation of fleas. Since the whole life history of the flea in Poona—where the weather is never cold—is conditioned in the same sense by atmospheric changes, it is clear that the numbers of the second generation are the product of the activity of the various phases. In July for example with a humidity of 79% and a temperature of 77°, 100 fleas lay 241 eggs of which 33% hatch; of the resulting 80 larvae, 20% develop successfully and the 16 fleas obtained, living on the average three days, would give 48 flea-days. In April, on the other hand, the humidity being 37% with a temperature of 84°, 100 fleas yielded only 88 eggs and these only 1.5 larvae: assuming that 1% of these developed, we get 0.015 fleas

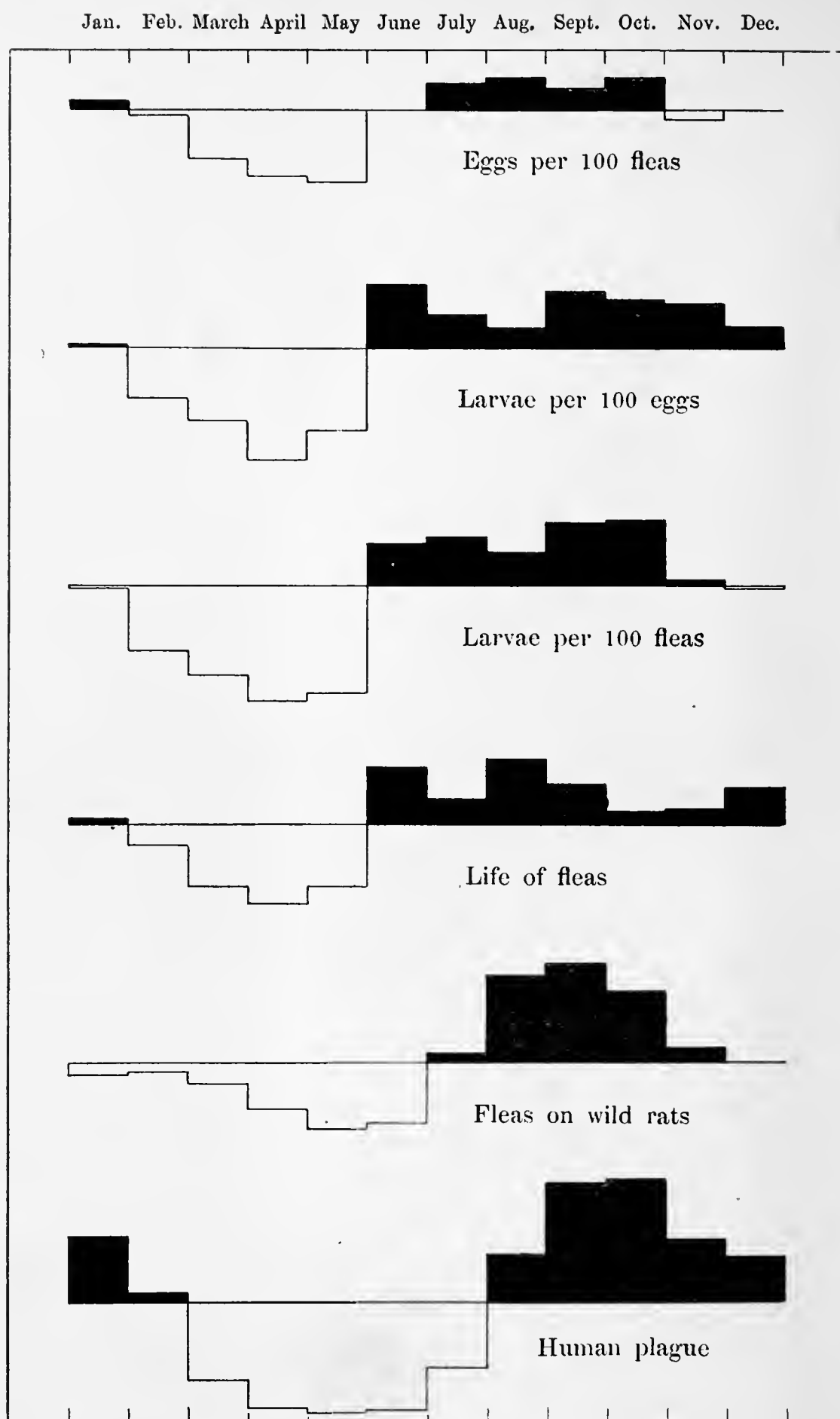


Fig. 7.

with an average life of 0·8 days giving a total life of 17 minutes or  $\frac{1}{4000}$ th of the July figure.

These figures cannot of course be transferred directly to the breeding of fleas under natural surroundings. Taken as a whole however they afford strong evidence that the seasonal variation in the prevalence of fleas, which has been shown to be one of the circumstances which condition the occurrence of epidemic plague, is due to factors which exercise a direct influence on the flea, and that among these factors are atmospheric humidity and, to a less extent within the range examined, temperature.

## LVI. THE SERUM TREATMENT OF HUMAN PLAGUE.

THE present series of observations on the treatment of plague with antisera has been carried out at the Maratha plague hospital, Bombay, during the epidemics from 1908—1911. Throughout the work for every case treated with serum a parallel case has been left without serum treatment; all cases received alike the ordinary hospital treatment. At first Dr Choksy, the medical officer in charge of the Maratha hospital, selected those which in his opinion were suitable for observation and the alternate cases received serum. Later on, however, when it became apparent that it was not possible on clinical grounds to differentiate the cases with grave, and as it turned out hopeless, septicaemia, every alternate case coming to hospital received serum, the moribund and those who had almost recovered alone being excluded from consideration.

A few cubic centimetres of blood were aseptically taken from a vein of each selected case. One quarter of a cubic centimetre was spread over the surface of an agar tube, and after incubation for 48 hours the cases could be divided into the following four groups:

- |     |  |         |
|-----|--|---------|
| (1) | blood sterile in $\frac{1}{4}$ c.c.            | = 0,    |
| (2) | 1 to 10 colonies from $\frac{1}{4}$ c.c.       | = +,    |
| (3) | 11 to 100 colonies from $\frac{1}{4}$ cc.      | = + +,  |
| (4) | more than 100 colonies from $\frac{1}{4}$ c.c. | = + + + |

Similar cultures were taken as far as possible from each patient every day till the patient either died or recovered: in this way the progress of the septicaemia could be observed.

Two kinds of serum were used: (1) the ordinary Yersin serum prepared by the injection of dead and afterwards living bacilli, made by the Lister Institute: (2) antitoxic serum from horses injected with the toxic nucleoproteid of the plague bacillus by Rowland (vol. XI. supplement, p. 11) and MacConkey (*infra*, p. 387) which was of proved efficacy in protecting rats from the injection of living broth cultures of plague bacilli. The serum was given in large doses, generally both intravenously and subcutaneously, sometimes subcutaneously only and

in a few cases intravenously only. In many cases further doses were given (usually subcutaneously) on succeeding days. In this way, as will be seen from table VI, a number of patients received altogether 500 c.c. of serum and upward.

The data concerning each case are shortly set out in table VI. In all 444 cases were observed, 222 being treated with serum and 222 being taken as controls. Table I summarises the results obtained:

TABLE I.

*Summary of cases grouped by the degree of septicaemia.*

Septicaemia	Serum			Control			Total	% died
	Died	Recovered	% died	Died	Recovered	% died		
0	22	63	25·9	24	46	34·3	155	29·7
+	35	12	74·5	35	12	74·5	94	74·5
++	15	0	100	24	0	100	39	100
+++	75	0	100	81	0	100	156	100
Total	147	75	66·2 %	164	58	73·9 %	444	70·0 %
	222			222				

From this it appears that, while 70 % of the cases without bacilli in the blood survived, three-quarters of those with a slight degree of septicaemia (+) died and none of the 195 with higher grades (++ and +++) recovered. The latter may therefore be excluded in the comparison between serum cases and controls. The percentage fatality in the + group is exactly the same whether serum was used or not, and it is only in the sterile group that any difference in the result is seen. Here, in the controls 24 out of 70 died or 34 %, while of the similar serum cases only 22 died out of 85 or 26 %. The difference therefore is in favour of the serum by some eight cases per hundred, but it is not large enough with such a small number of cases to indicate any definite influence of serum and does not satisfy the customary statistical test for error due to random sampling.

The next tables (II and III) show the same cases divided into two groups according to whether Yersin or antitoxic serum was used: in each case the controls belonging to the same period are given for comparison and the total controls for each group (which is probably the fairer figure) for comparison are given in table I.

TABLE II.

*Summary of cases treated with Yersin serum with the contemporary controls.*

Septicaemia	Serum			Controls			Total
	Died	Recovered	% died	Died	Recovered	% died	
0	14	43	24.6	10	31	24.4	98
+	22	8	73.3	32	10	76.2	72
++	12	0	100	16	0	100	28
+++	47	0	100	47	0	100	94
Total	95	51	65.1 %	105	41	71.9 %	292
	146			146			

TABLE III.

*Summary of cases treated with antitoxic serum with the contemporary controls.*

Septicaemia	Serum			Controls			Total
	Died	Recovered	% died	Died	Recovered	% died	
0	8	20	28.6	14	15	48.3	57
+	13	4	76.5	5	2	71.4	24
++	3	0	100	6	0	100	9
+++	28	0	100	34	0	100	62
Total	52	24	68.4 %	59	17	77.6 %	152
	76			76			

The Yersin series shows no indication of any advantage for the serum cases as against its own controls and the reduction of mortality (10%) in group 0 as compared with the total controls is of indefinite significance. In the antitoxic series, the mortality is a good deal less (20%) than in the contemporary controls but the difference is not statistically significant and the exceptionally high mortality among the controls indicates that they are not a fair sample.

In table IV is shown the average duration of illness in the relevant groups as indicated by the length of their stay in hospital. Making

TABLE IV.

*Average of days in hospital of cases which recovered.*

Septicaemia	Controls	Yersin serum	Antitoxic serum	Total serum
0	45	43	56	47
+	77	56	45	52



some allowance for the fact that the convalescence of the serum cases is often somewhat retarded by arthritis and other "serum phenomena," it appears that in group + the serum cases get better rather sooner than the controls. Otherwise the figures are negative.

Table V shows in the same way the duration of life in the fatal cases. As is the case with the percentage fatality (see table I), the length of life varies with the degree of septicaemia. In the grave cases (groups ++ and ++++) life appears to be prolonged by the use of serum but it is suspicious that this does not seem to be the case in those with no or a slight septicaemia.

TABLE V.

*Average length of life after admission to hospital of cases which died.*

Septicaemia	0	+	++	+++	Total
Control	7.4 *	5.2	2.9	2.0	3.6 days
Serum	9.2	5.3	5.3	2.7	4.6 days *

\* 13.1 if case 52, living 146 days, is included.

*From the whole enquiry therefore it appears that the administration of the available sera is not a practicable means of bringing about any material diminution in the mortality from plague in India. It may well be that better results would be obtained if the treatment could be commenced within a few hours of the onset of the disease: this however is, in the great majority of cases, impossible in ordinary hospital practice.*

We are glad to take this opportunity of expressing our indebtedness to Dr N. H. Choksy, without whose cordial encouragement and assistance this enquiry would have been hardly possible. Dr Choksy's own observations<sup>1</sup> on the serum treatment of plague have extended over many years and he has an unrivalled experience of the matter.

<sup>1</sup> See *British Medical Journal*, 1908, vol. I. p. 1282 and the references there given. The earlier observations in India are summarised by W. B. Bannerman in *Scientific Memoirs*, No. 20, 1905.

TABLE VI.

I. *Control cases without serum treatment.*

The fatal cases are shown in heavy type.

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
2	539	M	12	27. 4. 08	?	35	—	0
4	<b>542</b>	<b>M</b>	<b>15</b>	<b>27. 4. 08</b>	<b>2</b>	—	<b>4</b>	<b>++</b>
6	<b>548</b>	<b>M</b>	<b>25</b>	<b>28. 4. 08</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
8	551	F	15	23. 4. 08	2	48	—	0
10	<b>561</b>	<b>M</b>	<b>19</b>	<b>29. 4. 08</b>	<b>4</b>	—	<b>1</b>	<b>+++</b>
12	563	M	25	29. 4. 08	2	47	—	0
14	<b>567</b>	<b>M</b>	<b>25</b>	<b>29. 4. 08</b>	<b>2</b>	—	<b>4</b>	<b>+++</b>
16	571	M	12	30. 4. 08	3	31	—	0
18	<b>585</b>	<b>M</b>	<b>30</b>	<b>2. 5. 08</b>	<b>2</b>	—	<b>6</b>	<b>0</b>
20	588	M	35	3. 5. 08	4	42	—	0
22	<b>612</b>	<b>M</b>	<b>20</b>	<b>6. 5. 08</b>	<b>3</b>	—	<b>3</b>	<b>++ to +++</b>
24	615	M	[35]	6. 5. 08	3	52	—	+ to 0
26	<b>631</b>	<b>M</b>	<b>30</b>	<b>9. 5. 08</b>	<b>4</b>	—	<b>4</b>	<b>0</b>
28	649	M	16	12. 5. 08	3	34	—	+ to 0
30	<b>711</b>	<b>M</b>	<b>16</b>	<b>2. 6. 08</b>	<b>4</b>	—	<b>9</b>	<b>0</b>
32	712	M	20	2. 6. 08	4	36	—	0
34	130	M	30	15. 3. 09	3	116	—	+ to 0
36	<b>135</b>	<b>M</b>	<b>30</b>	<b>16. 3. 09</b>	<b>4</b>	—	<b>3</b>	<b>++</b>
38	<b>138</b>	<b>M</b>	<b>5</b>	<b>16. 3. 09</b>	<b>2</b>	—	<b>4</b>	<b>++</b>
40	<b>142</b>	<b>M</b>	<b>55</b>	<b>17. 3. 09</b>	<b>5</b>	—	<b>3</b>	<b>+++</b>
42	151	M	35	18. 3. 09	3	39	—	0
44	<b>158</b>	<b>M</b>	<b>16</b>	<b>19. 3. 09</b>	<b>2</b>	—	<b>4</b>	<b>+ to +++</b>
46	<b>160</b>	<b>M</b>	<b>32</b>	<b>19. 3. 09</b>	<b>3</b>	—	<b>6</b>	<b>+ to 0 to +</b>
48	161	M	30	19. 3. 09	5	68	—	0
50	<b>165</b>	<b>M</b>	<b>30</b>	<b>20. 3. 09</b>	<b>2</b>	—	<b>2</b>	<b>+++</b>
52	<b>167</b>	<b>M</b>	<b>35</b>	<b>20. 3. 09</b>	<b>3</b>	—	<b>146</b>	<b>0</b>
54	<b>170</b>	<b>M</b>	<b>20</b>	<b>20. 3. 09</b>	<b>1</b>	—	<b>3</b>	<b>+++</b>
56	<b>188</b>	<b>M</b>	<b>35</b>	<b>22. 3. 09</b>	<b>3</b>	—	<b>5</b>	<b>+ to +++</b>
58	179	F	27	22. 3. 09	1	28	—	0
60	<b>189</b>	<b>M</b>	<b>12</b>	<b>22. 3. 09</b>	<b>2</b>	—	<b>2</b>	<b>+++</b>
62	198	M	25	23. 3. 09	1	34	—	0
64	<b>201</b>	<b>M</b>	<b>25</b>	<b>24. 3. 09</b>	<b>4</b>	—	<b>4</b>	<b>+ to +++</b>
66	<b>205</b>	<b>M</b>	<b>15</b>	<b>24. 3. 09</b>	<b>2</b>	—	<b>7</b>	<b>+ to 0</b>
68	<b>212</b>	<b>M</b>	<b>30</b>	<b>25. 3. 09</b>	<b>4</b>	—	<b>4</b>	<b>+ to 0</b>
70	<b>218</b>	<b>F</b>	<b>10</b>	<b>26. 3. 09</b>	<b>1</b>	—	<b>5</b>	<b>+ to +++</b>
72	<b>225</b>	<b>M</b>	<b>20</b>	<b>27. 3. 09</b>	<b>2</b>	—	<b>4</b>	<b>++ to +++</b>
74	<b>237</b>	<b>M</b>	<b>35</b>	<b>29. 3. 09</b>	<b>2</b>	—	<b>1</b>	<b>+++</b>
76	239	M	30	29. 3. 09	4	42	—	0
78	<b>241</b>	<b>F</b>	<b>26</b>	<b>29. 3. 09</b>	<b>2</b>	—	<b>5</b>	<b>+ to +++</b>
80	<b>247</b>	<b>M</b>	<b>44</b>	<b>30. 3. 09</b>	<b>3</b>	—	<b>3</b>	<b>+ to +++</b>

TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
82	<b>249</b>	<b>F</b>	<b>35</b>	<b>30. 3. 09</b>	<b>2</b>	—	<b>13</b>	<b>+ to 0</b>
84	<b>271</b>	<b>M</b>	<b>55</b>	<b>3. 4. 09</b>	<b>2</b>	—	<b>1</b>	<b>+++</b>
86	275	M	16	4. 4. 09	2	43	—	+ to 0
88	276	F	35	4. 4. 09	3	29	—	0
90	280	M	16	5. 4. 09	2	42	—	0
92	<b>282</b>	<b>F</b>	<b>25</b>	<b>5. 4. 09</b>	<b>3</b>	—	<b>2</b>	<b>+++</b>
94	<b>299</b>	<b>M</b>	<b>18</b>	<b>8. 4. 09</b>	<b>3</b>	—	<b>1</b>	<b>+++</b>
96	<b>302</b>	<b>M</b>	<b>28</b>	<b>9. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+ to 0</b>
98	<b>304</b>	<b>M</b>	<b>10</b>	<b>9. 4. 09</b>	<b>2</b>	—	<b>1</b>	<b>+++</b>
100	<b>309</b>	<b>M</b>	<b>15</b>	<b>9. 4. 09</b>	<b>3</b>	—	<b>3</b>	<b>+ to +++</b>
102	<b>319</b>	<b>M</b>	<b>50</b>	<b>11. 4. 09</b>	<b>3</b>	—	<b>4</b>	<b>++ to +</b>
104	<b>327</b>	<b>M</b>	<b>16</b>	<b>13. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
106	<b>331</b>	<b>M</b>	<b>28</b>	<b>13. 4. 09</b>	<b>3</b>	—	<b>3</b>	<b>+++</b>
108	<b>337</b>	<b>F</b>	<b>30</b>	<b>14. 4. 09</b>	<b>3</b>	—	<b>4</b>	<b>+</b>
110	<b>344</b>	<b>M</b>	<b>28</b>	<b>16. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
112	<b>347</b>	<b>M</b>	<b>35</b>	<b>16. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>++</b>
114	349	F	18	16. 4. 09	2	52	—	0
116	<b>352</b>	<b>M</b>	<b>20</b>	<b>17. 4. 09</b>	<b>2</b>	—	<b>2</b>	<b>+++</b>
118	355	M	20	17. 4. 09	4	61	—	0
120	354	F	11	17. 4. 09	2	37	—	0
122	<b>362</b>	<b>M</b>	<b>25</b>	<b>18. 4. 09</b>	<b>2</b>	—	<b>5</b>	<b>+++</b>
124	368	F	25	19. 4. 09	2	21	—	0
126	<b>369</b>	<b>M</b>	<b>24</b>	<b>20. 4. 09</b>	<b>7</b>	—	<b>1</b>	<b>+++</b>
128	<b>372</b>	<b>M</b>	<b>35</b>	<b>20. 4. 09</b>	<b>2</b>	—	<b>5</b>	<b>+++</b>
130	<b>382</b>	<b>M</b>	<b>32</b>	<b>21. 4. 09</b>	<b>3</b>	—	<b>3</b>	<b>+ to ++</b>
132	<b>386</b>	<b>M</b>	<b>25</b>	<b>21. 4. 09</b>	<b>2</b>	—	<b>4</b>	<b>+ to +++</b>
134	392	F	5	22. 4. 09	4	81	—	+ to 0
136	<b>398</b>	<b>M</b>	<b>25</b>	<b>23. 4. 09</b>	<b>4</b>	—	<b>4</b>	<b>+++</b>
138	<b>406</b>	<b>F</b>	<b>9</b>	<b>23. 4. 09</b>	<b>4</b>	—	<b>3</b>	<b>+++</b>
140	<b>408</b>	<b>M</b>	<b>40</b>	<b>23. 4. 09</b>	<b>4</b>	—	<b>1</b>	<b>+++</b>
142	<b>409</b>	<b>M</b>	<b>60</b>	<b>24. 4. 09</b>	<b>3</b>	—	<b>1</b>	<b>+++</b>
144	<b>412</b>	<b>M</b>	<b>24</b>	<b>24. 4. 09</b>	<b>2</b>	—	<b>5</b>	<b>0</b>
146	<b>415</b>	<b>M</b>	<b>50</b>	<b>25. 4. 09</b>	<b>2</b>	—	<b>5</b>	<b>+ to +++</b>
148	<b>423</b>	<b>M</b>	<b>35</b>	<b>26. 4. 09</b>	<b>3</b>	—	<b>3</b>	<b>+++</b>
150	<b>428</b>	<b>M</b>	<b>12</b>	<b>26. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
152	429	M	17	27. 4. 09	4	50	—	+ to 0
154	<b>446</b>	<b>F</b>	<b>30</b>	<b>30. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
156	<b>448</b>	<b>M</b>	<b>23</b>	<b>30. 4. 09</b>	<b>2</b>	—	<b>1</b>	<b>++</b>
158	<b>469</b>	<b>M</b>	<b>19</b>	<b>2. 5. 09</b>	<b>1</b>	—	<b>4</b>	<b>+++</b>
160	<b>477</b>	<b>M</b>	<b>45</b>	<b>4. 5. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
162	<b>481</b>	<b>M</b>	<b>20</b>	<b>5. 5. 09</b>	<b>3</b>	—	<b>4</b>	<b>+ to +++</b>
164	498	F	32	10. 5. 09	7	84	—	0
166	<b>84</b>	<b>M</b>	<b>35</b>	<b>15. 3. 10</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>
168	91	M	30	16. 3. 10	4	53	—	0
170	<b>94</b>	<b>M</b>	<b>20</b>	<b>16. 3. 10</b>	<b>2</b>	—	<b>1</b>	<b>+++</b>

TABLE VI (contd.).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
172	98	M	25	18. 3. 10	4	—	3	+
174	102	M	11	20. 3. 10	4	—	7	+ to +++
176	105	M	30	20. 3. 10	2	—	2	++ to +++
178	110	M	40	23. 3. 10	3	—	3	+ to 0
180	112	M	14	23. 3. 10	4	—	3	+++
182	116	M	20	24. 3. 10	5	—	2	+++
184	120	M	28	26. 3. 10	2	51	—	0
186	126	M	35	30. 3. 10	4	—	2	+ to ++
188	129	M	25	30. 3. 10	2	—	2	+++
190	131	M	25	31. 3. 10	4	—	6	0
192	134	M	14	31. 3. 10	4	—	1	++
194	137	M	30	2. 4. 10	2	—	1	+++
196	144	M	18	5. 4. 10	4	—	1	++ to +++
198	153	M	25	7. 4. 10	5	108	—	0
200	168	M	30	13. 4. 10	3	—	1	+++
202	172	M	25	15. 4. 10	4	—	2	+ to ++
204	180	M	18	17. 4. 10	4	109	—	+ to 0
206	186	M	18	20. 4. 10	3	—	2	+++
208	191	M	14	21. 4. 10	2	15	—	0
210	197	M	30	22. 4. 10	6	59	—	0
212	199	M	40	22. 4. 10	4	46	—	+ to 0
214	205	M	45	25. 4. 10	2	—	7	+ to 0
216	210	M	50	27. 4. 10	3	26	—	0
218	216	F	35	28. 4. 10	4	—	2	++ to +++
220	227	M	20	30. 4. 10	2	—	2	+++
222	237	M	20	3. 5. 10	3	—	2	+++
224	243	M	40	3. 5. 10	2	62	—	0
226	246	F	35	5. 5. 10	5	—	2	+++
228	256	M	24	6. 5. 10	2	—	5	+ to 0
230	275	F	30	11. 5. 10	4	—	3	+++
232	277	M	35	12. 5. 10	4	—	6	0
234	290	M	30	13. 5. 10	3	—	4	+ to 0
236	294	M	25	14. 5. 10	5	73	—	0
238	19	M	22	30. 1. 11	3	—	1	+++
240	22	F	11	2. 2. 11	4	—	1	+++
242	25	M	30	6. 2. 11	4	—	4	0
244	30	M	20	8. 2. 11	4	—	7	+ to 0 to +
246	32	M	15	9. 2. 11	4	—	3	++
248	34	M	35	10. 2. 11	4	26	—	0
250	36	M	20	11. 2. 11	4	—	1	+++
252	43	M	30	16. 2. 11	4	61	—	+ to 0 to + to 0
254	49	F	35	19. 2. 11	4	—	13	0
256	54	M	20	20. 2. 11	4	—	3	+++
258	67	M	17	28. 2. 11	3	—	4	+ to +++
260	71	M	45	1. 3. 11	3	—	1	0

TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
262	84	M	25	6. 3. 11	5	—	5	0
264	90	M	25	7. 3. 11	2	—	1	0
266	92	M	30	8. 3. 11	5	68	—	0
268	93	M	17	7. 3. 11	3	—	1	+++
270	99	M	13	10. 3. 11	3	—	1	+++
272	105	M	19	11. 3. 11	5	—	3	++ to +++
274	107	F	45	11. 3. 11	5	45	—	0
276	110	M	20	13. 3. 11	3	—	2	+++
278	112	F	20	13. 3. 11	3	49	—	0
280	118	F	8	17. 3. 11	3	39	—	0
282	124	M	35	19. 3. 11	4	—	4	0
284	128	M	58	20. 3. 11	3	—	4	0
286	136	M	30	21. 3. 11	4	—	3	+++
288	137	M	30	21. 3. 11	3	—	1	+++
290	147	M	22	23. 3. 11	2	—	3	+++
292	150	M	50	24. 3. 11	3	—	4	++ to +++
294	157	M	30	25. 3. 11	3	51	—	0
296	161	M	16	27. 3. 11	3	—	1	+++
298	165	M	30	28. 3. 11	3	48	—	0
300	169	M	35	28. 3. 11	5	34	—	0
302	173	M	28	29. 3. 11	5	—	3	++
304	178	M	30	30. 3. 11	2	—	1	+++
306	180	M	22	30. 3. 11	3	—	6	0
308	187	M	25	31. 3. 11	3	—	1	+++
310	196	M	25	2. 4. 11	2	29	—	0
312	204	F	40	3. 4. 11	2	28	—	0
314	207	M	18	4. 4. 11	4	—	3	+++
316	209	M	25	4. 4. 11	4	—	1	+++
318	218	F	20	5. 4. 11	4	33	—	0
320	220	M	16	5. 4. 11	6	20	—	0
322	222	M	22	5. 4. 11	4	—	8	+ to 0
324	226	M	30	5. 4. 11	4	—	2	+++
326	230	M	30	6. 4. 11	4	—	4	++
328	235	F	12	7. 4. 11	3	38	—	+ to 0
330	247	M	50	8. 4. 11	5	—	3	0
332	252	M	60	8. 4. 11	2	—	5	++
334	254	M	40	9. 4. 11	3	—	4	+++
336	257	M	16	9. 4. 11	4	—	4	+++
338	264	F	18	10. 4. 11	3	—	9	0 to +
340	260	M	30	10. 4. 11	3	—	2	+++
342	268	M	28	11. 4. 11	5	—	1	+++
344	271	M	35	11. 4. 11	2	—	1	+++
346	272	F	45	12. 4. 11	3	—	1	+++
348	275	M	25	12. 4. 11	5	—	16	0
350	277	M	12	12. 4. 11	3	—	8	0

TABLE VI (contd.).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
352	280	M	30	12. 4. 11	4	—	8	0
354	283	M	30	12. 4. 11	2	—	2	+++
356	290	F	12	13. 4. 11	5	32	—	0
358	298	M	30	15. 4. 11	3	—	3	+++
360	300	M	22	15. 4. 11	3	—	3	+++
362	304	M	55	16. 4. 11	2	—	1	+++
364	309	M	30	17. 4. 11	5	—	1	+++
366	315	M	20	18. 4. 11	3	—	1	+++
368	319	M	22	18. 4. 11	3	—	4	+++
370	320	M	40	18. 4. 11	5	—	1	+++
372	322	M	25	20. 4. 11	3	—	5	+ to +++
374	324	M	60	19. 4. 11	3	—	3	+++
376	329	M	25	20. 4. 11	5	61	—	0
378	333	M	30	21. 4. 11	5	72	—	0
380	335	F	14	21. 4. 11	4	—	5	+ to 0 to +
382	337	M	45	21. 4. 11	4	—	1	+++
384	342	M	22	22. 4. 11	5	—	1	+++
386	344	M	20	22. 4. 11	5	—	6	0
388	347	M	25	22. 4. 11	4	—	1	+++
390	350	F	30	24. 4. 11	4	—	4	0
392	356	F	22	25. 4. 11	3	—	7	+ to 0
394	358	M	20	27. 4. 11	5	—	1	0
396	367	M	25	27. 4. 11	2	—	10	+ to 0
398	369	F	20	27. 4. 11	2	43	—	0
400	372	M	30	28. 4. 11	3	—	2	+++
402	376	M	10	28. 4. 11	2	—	1	+++
404	381	M	30	28. 4. 11	2	64	—	0
406	385	M	22	29. 4. 11	3	—	3	++
408	387	M	40	29. 4. 11	3	—	1	+++
410	394	M	22	1. 5. 11	3	—	1	++
412	401	M	30	3. 5. 11	4	70	—	0
414	409	M	22	4. 5. 11	4	—	1	+++
416	411	M	18	4. 5. 11	3	—	2	+++
418	417	M	40	6. 5. 11	3	—	1	+++
420	422	M	38	7. 5. 11	5	—	4	++
422	423	M	16	8. 5. 11	5	—	40	0
424	432	M	22	8. 5. 11	2	—	5	+
426	438	M	14	10. 5. 11	2	—	4	++ to + to ++
428	442	F	14	11. 5. 11	3	—	4	+++
430	445	M	12	13. 5. 11	3	23	—	0
432	447	M	12	14. 5. 11	3	172	—	+ to 0
434	460	M	35	16. 5. 11	6	—	1	++
436	462	F	35	16. 5. 11	5	—	5	+ to 0 to +
438	467	M	25	17. 5. 11	3	—	3	++
440	470	F	18	22. 5. 11	3	90	—	+ to 0
442	476	M	20	24. 5. 11	2	—	13	+ to 0
444	479	M	12	25. 5. 11	6	68	—	+ to 0

TABLE VI (contd.).

II. Cases treated with serum.

A. Treated with Yersin's serum.

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c.	
									subcut.	intraven
1	540	M	25	27. 4. 08	3	35	—	0	—	180
3	541	M	40	27. 4. 08	1	—	2	+++	—	100
5	547	M	25	28. 4. 08	1	—	3	++	—	95
7	554	F	40	28. 4. 08	4	35	—	0	—	100
9	557	M	13	29. 4. 08	2	—	3	+++	—	100
11	560	M	20	29. 4. 08	4	—	3	++	—	150
13	568	M	20	29. 4. 08	1	—	3	+++	—	50
15	569	M	30	29. 4. 08	2	—	8	+ to 0	—	610
17	572	F	20	30. 4. 08	3	—	4	++	—	150
19	586	M	22	3. 5. 08	3	—	3	++ to +++	—	200
21	589	M	15	3. 5. 08	2	43	—	+ to 0	100	400
23	613	M	29	6. 5. 08	4	—	7	0	320	400
25	632	F	55	9. 5. 08	4	48	—	0	160	300
27	637	M	40	9. 5. 08	2	—	25	0	220	200
29	713	M	16	2. 6. 08	2	42	—	0	90	100
31	714	M	40	3. 6. 08	3	—	1	+++	100	100
33	133	M	23	15. 3. 09	4	77	—	0	320	—
35	136	M	30	16. 3. 09	2	69	—	+ to 0	220	210
37	141	M	20	17. 3. 09	6	27	—	0	100	—
39	146	M	30	18. 3. 09	4	—	3	+++	160	170
41	150	M	35	18. 3. 09	2	39	—	0	220	—
43	152	F	45	18. 3. 09	2	52	—	0	360	—
45	155	M	55	18. 3. 09	1	—	6	++ to + to ++	220	140
47	162	M	22	19. 3. 09	2	52	—	+ to 0	320	220
49	164	M	18	20. 3. 09	3	—	2	+++	160	160
51	166	M	18	20. 3. 09	2	44	—	0	280	280
53	169	F	23	20. 3. 09	3	—	3	+++	160	120
55	173	M	30	21. 3. 09	3	—	1	+++	160	170
57	183	M	20	22. 3. 09	1	56	—	0	520	180
59	186	M	30	22. 3. 09	3	—	4	+	220	220
61	192	F	15	22. 3. 09	1	37	—	0	360	160
63	200	M	25	24. 3. 09	5	—	11	0	500	100
65	202	M	16	24. 3. 09	2	—	8	++ to 0 to ++	600	—
67	206	M	57	25. 3. 09	4	—	3	+++ to ++	220	160
69	210	M	6	25. 3. 09	1	35	—	0	280	—
71	219	M	12	27. 3. 09	3	—	11	+ to 0 to +	560	—
73	231	F	12	28. 3. 09	3	—	2	0	100	100
75	238	M	20	29. 3. 09	4	41	—	0	260	100
77	245	M	12	30. 3. 09	4	—	1	+++	100	100
79	246	F	25	30. 3. 09	3	—	30	+ to 0	580	220

TABLE VI (contd.).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c.	
									subcut.	intraven.
81	254	M	40	31. 3. 09	2	33	—	0	220	160
83	258	M	25	1. 4. 09	3	39	—	0	160	80
85	<b>277</b>	<b>F</b>	<b>20</b>	<b>4. 4. 09</b>	<b>4</b>	—	<b>6</b>	<b>+++ to 0 to +</b>	<b>500</b>	—
87	278	M	25	4. 4. 09	1	43	—	0	320	165
89	<b>279</b>	<b>M</b>	<b>30</b>	<b>4. 4. 09</b>	<b>5</b>	—	<b>1</b>	<b>0</b>	<b>100</b>	<b>100</b>
91	<b>281</b>	<b>M</b>	<b>30</b>	<b>5. 4. 09</b>	<b>3</b>	—	<b>3</b>	<b>+++</b>	<b>100</b>	<b>100</b>
93	293	M	50	8. 4. 09	3	39	—	+ to 0	280	120
95	306	M	18	9. 4. 09	5	45	—	0	220	140
97	307	M	21	9. 4. 09	3	39	—	0	380	280
99	308	M	25	9. 4. 09	2	68	—	+ to 0	280	280
101	318	M	15	11. 4. 09	2	36	—	0	220	160
103	<b>322</b>	<b>F</b>	<b>28</b>	<b>11. 4. 09</b>	<b>2</b>	—	<b>2</b>	<b>+++</b>	<b>200</b>	—
105	<b>325</b>	<b>F</b>	<b>28</b>	<b>13. 4. 09</b>	<b>3</b>	—	<b>7</b>	<b>+++ to + to +++</b>	<b>160</b>	<b>560</b>
107	<b>328</b>	<b>M</b>	<b>30</b>	<b>13. 4. 09</b>	<b>2</b>	—	<b>2</b>	<b>+++</b>	<b>100</b>	<b>100</b>
109	<b>340</b>	<b>M</b>	<b>39</b>	<b>15. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>	<b>100</b>	<b>100</b>
111	345	M	26	16. 4. 09	2	31	—	0	160	160
113	350	F	15	16. 4. 09	1	24	—	0	140	80
115	<b>351</b>	<b>M</b>	<b>30</b>	<b>16. 4. 09</b>	<b>2</b>	—	<b>1</b>	<b>+++</b>	<b>100</b>	<b>100</b>
117	<b>353</b>	<b>M</b>	<b>15</b>	<b>17. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++ to 0</b>	<b>360</b>	<b>120</b>
119	<b>357</b>	<b>M</b>	<b>20</b>	<b>18. 4. 09</b>	<b>3</b>	—	<b>3</b>	<b>+++</b>	<b>200</b>	<b>60</b>
121	<b>359</b>	<b>M</b>	<b>30</b>	<b>18. 4. 09</b>	<b>5</b>	—	<b>3</b>	<b>++ to +</b>	<b>240</b>	<b>160</b>
123	364	M	24	19. 4. 09	2	35	—	0	300	100
125	<b>367</b>	<b>F</b>	<b>19</b>	<b>19. 4. 09</b>	<b>2</b>	—	<b>1</b>	<b>+</b>	<b>100</b>	<b>100</b>
127	<b>371</b>	<b>M</b>	<b>18</b>	<b>20. 4. 09</b>	<b>3</b>	—	<b>9</b>	<b>+ to 0</b>	<b>320</b>	<b>220</b>
129	374	M	30	20. 4. 09	1	33	—	0	360	100
131	<b>390</b>	<b>M</b>	<b>12</b>	<b>22. 4. 09</b>	<b>4</b>	—	<b>1</b>	<b>+++</b>	<b>60</b>	<b>40</b>
133	<b>394</b>	<b>F</b>	<b>24</b>	<b>22. 4. 09</b>	<b>3</b>	—	<b>4</b>	<b>+++</b>	<b>220</b>	<b>220</b>
135	<b>395</b>	<b>M</b>	<b>35</b>	<b>22. 4. 09</b>	<b>2</b>	—	<b>2</b>	<b>0</b>	<b>100</b>	<b>100</b>
137	<b>399</b>	<b>M</b>	<b>42</b>	<b>23. 4. 09</b>	<b>1</b>	—	<b>8</b>	<b>++ to 0</b>	<b>420</b>	<b>220</b>
139	<b>401</b>	<b>M</b>	<b>30</b>	<b>23. 4. 09</b>	<b>3</b>	—	<b>5</b>	<b>++ to + to ++</b>	<b>320</b>	<b>220</b>
141	<b>402</b>	<b>F</b>	<b>22</b>	<b>23. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>0</b>	<b>100</b>	<b>100</b>
143	<b>411</b>	<b>M</b>	<b>14</b>	<b>24. 4. 09</b>	<b>3</b>	—	<b>5</b>	<b>+++ to + to ++</b>	<b>140</b>	<b>100</b>
145	<b>414</b>	<b>M</b>	<b>32</b>	<b>25. 4. 09</b>	<b>3</b>	—	<b>4</b>	<b>+++ to ++ to +</b>	<b>160</b>	<b>140</b>
147	<b>422</b>	<b>M</b>	<b>22</b>	<b>26. 4. 09</b>	<b>3</b>	—	<b>6</b>	<b>+++ to +</b>	<b>160</b>	<b>120</b>
149	<b>424</b>	<b>F</b>	<b>9</b>	<b>26. 4. 09</b>	<b>2</b>	—	<b>3</b>	<b>+++</b>	<b>60</b>	<b>60</b>
151	<b>430</b>	<b>M</b>	<b>30</b>	<b>27. 4. 09</b>	<b>2</b>	—	<b>12</b>	<b>+++ to 0 to +</b>	<b>200</b>	<b>60</b>
153	<b>436</b>	<b>F</b>	<b>30</b>	<b>28. 4. 09</b>	<b>2</b>	—	<b>6</b>	<b>+++ to + to +++</b>	<b>160</b>	<b>120</b>
155	<b>440</b>	<b>F</b>	<b>40</b>	<b>29. 4. 09</b>	<b>3</b>	—	<b>4</b>	<b>0 to + to 0</b>	<b>300</b>	—
157	<b>447</b>	<b>F</b>	<b>14</b>	<b>30. 4. 09</b>	<b>2</b>	—	<b>1</b>	<b>0</b>	<b>60</b>	<b>60</b>
159	<b>462</b>	<b>F</b>	<b>25</b>	<b>2. 5. 09</b>	<b>2</b>	—	<b>6</b>	<b>+++ to +</b>	<b>100</b>	<b>60</b>
161	<b>465</b>	<b>M</b>	<b>32</b>	<b>2. 5. 09</b>	<b>3</b>	—	<b>1</b>	<b>+++</b>	<b>100</b>	<b>60</b>
163	<b>483</b>	<b>M</b>	<b>65</b>	<b>5. 5. 09</b>	<b>3</b>	—	<b>5</b>	<b>0</b>	<b>320</b>	—
165	<b>83</b>	<b>M</b>	<b>25</b>	<b>15. 3. 10</b>	<b>5</b>	—	<b>4</b>	<b>+ to 0</b>	<b>400</b>	<b>150</b>
167	87	M	30	15. 3. 10	4	75	—	+ to 0	550	200
169	<b>92</b>	<b>M</b>	<b>38</b>	<b>16. 3. 10</b>	<b>4</b>	—	<b>6</b>	<b>+ to 0</b>	<b>450</b>	<b>115</b>
171	97	F	7	18. 3. 10	2	51	—	0	180	—



TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c.	
									subcut.	intraven.
173	99	M	25	18. 3. 10	3	—	5	+++ to ++	300	150
175	101	M	16	18. 3. 10	3	—	4	+++ to ++	400	100
177	107	M	35	20. 3. 10	4	—	1	+++	200	100
179	111	M	45	22. 3. 10	3	—	2	+++	100	50
181	114	M	20	23. 3. 10	5	—	4	+++	300	150
183	118	M	30	24. 3. 10	3	—	1	+++	150	—
185	127	M	28	30. 3. 10	4	40	—	0	520	150
187	128	M	16	30. 3. 10	2	40	—	0	520	50
189	132	M	28	31. 3. 10	3	—	7	+ to 0	580	150
191	133	M	25	31. 3. 10	4	—	5	+++ to +	300	150
193	136	M	30	1. 4. 10	2	—	1	+++	300	150
195	143	M	25	3. 4. 10	3	—	3	+++	300	150
197	151	M	10	5. 4. 10	1	22	—	0	100	50
199	167	M	30	13. 4. 10	4	42	—	0	200	100
201	171	M	18	15. 4. 10	4	—	4	+ to ++	400	150
203	178	M	44	16. 4. 10	2	—	11	+ to 0	720	45
205	184	M	20	19. 4. 10	3	62	—	0	500	150
207	190	M	20	20. 4. 10	4	41	—	0	400	150
209	193	M	35	21. 4. 10	2	39	—	+ to 0	400	150
211	198	M	35	22. 4. 10	3	—	2	+++	300	150
213	204	M	30	25. 4. 10	3	92	—	0	500	200
215	207	M	18	26. 4. 10	2	—	7	+ to 0	560	150
217	212	M	30	27. 4. 10	2	—	7	+ to 0 to ++	560	150
219	222	M	20	30. 4. 10	2	21	—	0	460	150
221	235	M	16	3. 5. 10	5	—	6	+ to 0	300	150
223	242	M	15	3. 5. 10	1	34	—	0	400	150
225	244	F	35	5. 5. 10	5	47	—	0	460	150
227	255	M	16	6. 5. 10	3	—	1	+++	300	—
229	268	F	27	10. 5. 10	3	—	1	0	200	100
231	282	M	28	11. 5. 10	3	26	—	0	300	150
233	291	M	25	14. 5. 10	4	—	3	+ to 0	500	25
235	289	M	25	13. 5. 10	3	54	—	0	450	—
387	343	M	20	22. 4. 11	5	—	2	+++	200	80
391	349	M	20	23. 4. 11	4	—	1	+++	100	40
393	355	M	15	25. 4. 11	4	35	—	0	400	120
395	362	M	58	26. 4. 11	3	54	—	0	400	80
397	366	M	15	27. 4. 11	4	—	16	0	400	80
399	368	M	20	27. 4. 11	2	—	1	+	100	40
401	370	M	22	28. 4. 11	3	—	3	+++	200	80
403	374	F	30	28. 4. 11	4	—	7	+ to 0	560	80
405	380	M	22	28. 4. 11	4	—	46	0	400	80
407	382	M	32	28. 4. 11	3	—	3	+++	200	80
409	386	M	33	29. 4. 11	4	44	—	0	600	80
411	391	M	29	29. 4. 11	3	—	3	+ to 0	200	80
413	396	M	12	1. 5. 11	3	—	7	++ to 0 to +	500	20
415	406	M	38	4. 5. 11	4	—	1	0	100	40

TABLE VI (contd.).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c.	
									subcut.	intraven.
417	<b>414</b>	<b>M</b>	<b>18</b>	<b>5. 5. 11</b>	<b>3</b>	—	<b>1</b>	+++	<b>100</b>	<b>40</b>
419	<b>421</b>	<b>M</b>	<b>30</b>	<b>7. 5. 11</b>	<b>4</b>	—	<b>1</b>	+++	<b>100</b>	<b>40</b>
421	427	M	30	7. 5. 11	5	55	—	0	400	80
423	<b>428</b>	<b>M</b>	<b>24</b>	<b>7. 5. 11</b>	<b>2</b>	—	<b>5</b>	++ to +	<b>400</b>	<b>80</b>
425	439	M	20	10. 5. 11	2	68	—	0	260	80
427	444	M	32	12. 5. 11	3	66	—	0	300	80
429	446	M	30	13. 5. 11	6	60	—	+ to 0	500	120
431	<b>449</b>	<b>M</b>	<b>14</b>	<b>14. 5. 11</b>	<b>2</b>	—	<b>3</b>	++	<b>300</b>	<b>80</b>
433	<b>461</b>	<b>M</b>	<b>20</b>	<b>16. 5. 11</b>	<b>4</b>	—	<b>2</b>	+	<b>200</b>	<b>80</b>
435	<b>463</b>	<b>F</b>	<b>25</b>	<b>16. 5. 11</b>	<b>5</b>	—	<b>19</b>	+ to 0	<b>500</b>	<b>100</b>
437	468	M	30	19. 5. 11	3	44	—	0	300	80
439	<b>471</b>	<b>M</b>	<b>45</b>	<b>22. 5. 11</b>	<b>3</b>	—	<b>4</b>	+	<b>260</b>	<b>40</b>
441	<b>477</b>	<b>M</b>	<b>21</b>	<b>24. 5. 11</b>	<b>3</b>	—	<b>2</b>	+++	<b>200</b>	<b>80</b>
443	<b>485</b>	<b>M</b>	<b>30</b>	<b>28. 5. 11</b>	<b>3</b>	—	<b>1</b>	+	<b>100</b>	<b>40</b>

*B. Treated with antitoxic serum.*

237	<b>8</b>	<b>M</b>	<b>16</b>	<b>21. 1. 11</b>	<b>3</b>	—	<b>1</b>	0	<b>100</b>	<b>25</b>
239	20	M	27	30. 1. 11	1	57	—	0	240	40
241	23	F	53	2. 2. 11	5	91	—	0	180	40
243	27	F	16	7. 2. 11	4	67	—	0	240	—
245	<b>29</b>	<b>F</b>	<b>20</b>	<b>7. 2. 11</b>	<b>4</b>	—	<b>4</b>	++ to 0 to +	<b>240</b>	<b>60</b>
247	31	M	18	8. 2. 11	4	76	—	0	240	—
249	<b>33</b>	<b>F</b>	<b>45</b>	<b>9. 2. 11</b>	<b>6</b>	—	<b>8</b>	+ to 0 to ++	<b>100</b>	<b>40</b>
251	35	M	30	10. 2. 11	4	66	—	0	120	40
253	37	F	35	11. 2. 11	3	72	—	0	300	40
255	44	F	30	16. 2. 11	4	61	—	0	380	100
257	65	M	40	27. 2. 11	4	63	—	0	400	80
259	<b>70</b>	<b>M</b>	<b>30</b>	<b>1. 3. 11</b>	<b>6</b>	—	<b>4</b>	+ to +++	<b>300</b>	<b>120</b>
261	72	F	21	1. 3. 11	2	34	—	+ to 0	500	120
263	<b>87</b>	<b>M</b>	<b>16</b>	<b>6. 3. 11</b>	<b>2</b>	—	<b>8</b>	0	<b>600</b>	<b>120</b>
265	<b>91</b>	<b>F</b>	<b>30</b>	<b>7. 3. 11</b>	<b>4</b>	—	<b>10</b>	0	<b>660</b>	<b>120</b>
267	94	M	12	8. 3. 11	3	68	—	0	560	120
269	<b>97</b>	<b>M</b>	<b>18</b>	<b>9. 3. 11</b>	<b>4</b>	—	<b>4</b>	++ to +++	<b>400</b>	<b>80</b>
271	<b>101</b>	<b>M</b>	<b>35</b>	<b>10. 3. 11</b>	<b>4</b>	—	<b>1</b>	+++	<b>100</b>	<b>40</b>
273	<b>106</b>	<b>M</b>	<b>22</b>	<b>11. 3. 11</b>	<b>2</b>	—	<b>10</b>	0	<b>460</b>	<b>80</b>
275	<b>108</b>	<b>F</b>	<b>30</b>	<b>12. 3. 11</b>	<b>3</b>	—	<b>1</b>	+++	<b>100</b>	<b>40</b>
277	<b>111</b>	<b>F</b>	<b>40</b>	<b>13. 3. 11</b>	<b>2</b>	—	<b>9</b>	0	<b>400</b>	<b>120</b>
279	113	M	23	13. 3. 11	3	68	—	0	460	80
281	<b>122</b>	<b>M</b>	<b>35</b>	<b>19. 3. 11</b>	<b>4</b>	—	<b>1</b>	+++	<b>100</b>	<b>40</b>
283	<b>127</b>	<b>M</b>	<b>20</b>	<b>19. 3. 11</b>	<b>3</b>	—	<b>4</b>	+ to 0 to +	<b>300</b>	<b>40</b>
285	<b>132</b>	<b>M</b>	<b>30</b>	<b>20. 3. 11</b>	<b>5</b>	—	<b>3</b>	+++	<b>200</b>	<b>40</b>
287	<b>134</b>	<b>M</b>	<b>29</b>	<b>20. 3. 11</b>	<b>3</b>	—	<b>4</b>	+ to 0 to ++	<b>400</b>	<b>100</b>
289	<b>144</b>	<b>M</b>	<b>24</b>	<b>22. 3. 11</b>	<b>3</b>	—	<b>5</b>	+++	<b>400</b>	<b>100</b>
291	<b>148</b>	<b>M</b>	<b>35</b>	<b>23. 3. 11</b>	<b>2</b>	—	<b>4</b>	+++	<b>300</b>	<b>120</b>
293	151	M	30	24. 3. 11	5	59	—	0	260	80
295	<b>159</b>	<b>M</b>	<b>20</b>	<b>26. 3. 11</b>	<b>5</b>	—	<b>4</b>	+++	<b>300</b>	<b>100</b>

TABLE VI (contd.).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c.	
									subcut.	intraven.
297	163	M	17	27. 3. 11	3	—	5	+++	400	120
299	168	M	30	28. 3. 11	3	—	1	+++	100	40
301	170	F	20	28. 3. 11	3	34	—	0	400	40
303	172	F	22	28. 3. 11	2	—	1	+++	100	40
305	176	F	4	29. 3. 11	3	—	1	+++	40	—
307	185	M	20	31. 3. 11	2	—	5	+ to 0	300	120
309	189	F	50	31. 3. 11	3	18	—	0	300	80
311	195	M	20	31. 3. 11	2	—	1	+	100	40
313	205	M	30	4. 4. 11	3	51	—	0	500	120
315	208	M	50	4. 4. 11	5	—	8	+	500	120
317	210	M	16	4. 4. 11	3	—	1	+++	100	40
319	214	M	30	5. 4. 11	3	—	5	+ to +++	400	120
321	219	F	25	5. 4. 11	5	—	3	+++	200	80
323	221	M	26	5. 4. 11	2	—	1	+	100	40
325	223	M	15	5. 4. 11	5	55	—	+ to 0	600	80
327	229	M	24	6. 4. 11	1	—	6	0	500	120
329	234	M	15	7. 4. 11	4	38	—	0	400	120
331	238	F	22	7. 4. 11	3	—	1	+++	100	40
333	243	M	14	7. 4. 11	4	73	—	0	400	120
335	251	M	25	8. 4. 11	4	—	1	+++	100	40
337	253	M	12	9. 4. 11	3	—	1	+++	100	40
339	256	M	20	9. 4. 11	3	—	4	+++	400	120
341	261	M	30	10. 4. 11	2	57	—	0	400	120
343	266	M	30	10. 4. 11	1	19	—	0	260	80
345	265	F	40	10. 4. 11	3	—	5	+	300	120
347	262	M	28	10. 4. 11	4	30	—	0	300	80
349	267	M	33	10. 4. 11	2	—	1	+++	200	80
351	273	M	30	12. 4. 11	3	—	2	+++	100	40
353	276	M	15	12. 4. 11	3	55	—	0	400	120
355	297	F	31	12. 4. 11	3	—	11	0	500	80
357	282	M	15	12. 4. 11	5	—	4	+ to 0	400	80
359	284	M	23	13. 4. 11	3	—	1	+++	100	40
361	294	M	23	14. 4. 11	3	—	3	+++	200	80
363	299	M	25	15. 4. 11	3	—	4	+++	300	80
365	302	M	52	16. 4. 11	6	—	4	+ to 0	360	80
367	306	M	17	16. 4. 11	3	—	4	+++	200	80
369	312	M	22	17. 4. 11	4	—	1	++	200	80
371	316	M	30	18. 4. 11	3	—	1	+++	100	40
373	318	M	57	18. 4. 11	4	—	4	+	300	—
375	323	M	22	19. 4. 11	3	48	—	+ to 0	300	80
377	328	M	30	20. 4. 11	4	44	—	+ to 0	400	40
379	331	M	22	20. 4. 11	2	—	1	+++	200	40
381	332	M	25	20. 4. 11	5	—	1	+++	100	40
383	338	M	40	21. 4. 11	5	—	1	+++	100	40
385	341	M	20	21. 4. 11	3	—	19	0	500	40
389	345	M	20	22. 4. 11	3	—	1	+++	100	40

LVII. ATTEMPT TO SEPARATE THE ANTIGEN FROM THE  
NUCLEOPROTEIN OF THE PLAGUE BACILLUS BY  
FILTRATION THROUGH GELATIN.

By SYDNEY ROWLAND, M.A., M.R.C.S.

*Of the Lister Institute.*

THE antigen of plague as we have established in the course of these reports is intimately associated with a nucleoprotein soluble in dilute salines. By extracting this nucleoprotein from the bacillus the contained antigen (for rats) can be obtained. The relations that exist between the amount of antigen contained in the bacillus and the amount of antigen associated with the nucleoprotein extractable from the bacillus show that in the process of extraction there is little or no loss. The process is, within the limits of experimental error imposed on all measurements based on animal experiments, a quantitative one. In this respect it differs from the process of Lustig and Galeotti<sup>1</sup>. These observers used a 0.75% solution of NaOH as an extraction medium and the quantity of nucleoprotein obtained by their method necessary to immunise a rat is given by them as 0.36 mg. If this amount be compared with the amount of nucleoprotein associated with one protecting dose of the extract obtained by the sulphate of soda method, a striking contrast is apparent. A dose of extract containing 0.0001 mg. protects about 60 per cent. of the rats which receive it (*Journal of Hygiene*, vol. x. (1910), p. 559).

Not only is the associated quantity of nucleoprotein much smaller when the sulphate process is employed, but as far as can be seen there is no loss of antigen in the process. Thus the relations by weight of the nucleoprotein to the bacillus (both weighed dry) are as about ten to one, *i.e.*, from one gram of dry bacilli about 0.1 gram of nucleoprotein is obtained. The protective values of the bacillus and the nucleoprotein obtained from it are about the same. Thus the

<sup>1</sup> *Deutsch. med. Woch.* 1897, pp. 23, 227, 289.

mortality after the injection of rats with 0.03 mg. of whole bacillus is about 25%, while the mortality after injecting 0.001 mg. nucleoprotein is about 12%, the mortalities in both cases occurring after the injection into the test animals of the same quantity of the same virulent broth culture. It is very difficult to obtain exact experimental proof that no loss of antigen occurs on extraction; the figures just quoted show that there is no appreciable loss. In 0.03 mg. of whole bacillus there is 0.003 mg. of nucleoprotein. Inoculation of one-third of this amount was followed by a better immunity than followed the inoculation of 0.03 mg. of whole bacillus.

The question of the relations that exist between the nucleoprotein and the antigen might be elucidated by a study of the effects of filtering the nucleoprotein solution through gelatin, as the gelatin filter serves as a ready means of removing proteins from their solutions. Thus it would be possible to ascertain whether the antigen was so closely bound to the nucleoprotein that gelatin might or might not effect a separation more or less complete between them. More especially was this thought to be worth investigating in view of the results obtained by hydrolysing the nucleoprotein.

Hydrolysis was found to affect the nucleoprotein whilst leaving the antigen intact (vol. XI. (1911), supplement, p. 35). It was possible, therefore, that one of the effects of hydrolysis was to effect a separation between the antigen and the nucleoprotein. The data required are (1) the nucleoprotein content and antigenic value of a solution: (2) the same figures with regard to the same solution after passing through gelatin. With a view to obtaining information on this question the following experiments were carried out.

A solution was prepared. Its antigenic value is given by the experiments in the following table:

Dose	No. rats	No. survived	% survived
0.01 mg.	14	12	86

Thus 0.01 mg. protected 86% of the rats inoculated with it.

The solution was then allowed to autolyse for a month. It contained originally 2 mg. nucleoprotein per cubic centimetre. It was then filtered through 10% gelatin under a pressure of 40 atmospheres. The particular method employed was that devised by Martin<sup>1</sup>.

Under this pressure filtration was rapid and it was feared that the gelatin was not effecting a complete separation between the proteins

<sup>1</sup> *Journal of Physiology*, vol. xx. (1896) p. 364.

and the solution. The solution was accordingly passed through the filter several times until a small portion of the filtrate gave no reaction on boiling after acidification with acetic acid. The apparent passage of the nucleoprotein through the gelatin might be used as an argument that the constituent molecular aggregates are small. It must be remembered that the solution was, considered as a proteid solution, very dilute. It contained originally only 2 mg. per c.c. and after the month's lysis it contained less. Thus the solution was only 0·2%. The statements that are given as to the impermeability of 10% gelatin to protein solutions are all based on the examination of much stronger solutions than this. It is possible that very dilute solutions of all proteins pass through gelatin. Indeed, the fact that continued passage of the dilute solution used in this experiment did result in a more nearly complete arrest of the protein points to this conclusion.

After the filtration was complete a further test was made for the presence of protein. It was found that in 100 c.c. an amount of protein approximating to 1 mg. was present.

It now remained to determine the vaccinating value of this solution. The experiments performed to ascertain this point are given in the following table :

Dose	No. rats	No. survived	% survived
1 c.c.	19	16	84

The quantity of nucleoprotein contained in the vaccinating dose was 0·01 mg.

The amount of protection—84%—is practically identical with the protection obtained by the use of the solution before hydrolysis.

Now the hydrolysis has increased the ratio of antigen to nucleoprotein, for it has diminished the amount of protein recoverable by boiling in the presence of acetic acid (*Journal of Hygiene*, vol. XI. (1911), supplement, p. 27), and it has not diminished the amount of antigen (*loc. cit.*). We should expect, therefore, that even if the gelatin has not diminished the ratio of protein to antigen, the filtrate would show a high immunising value. The experiment quoted does not determine the ratio of antigen to protein. The protection afforded is high—over 80%. It was possible that a smaller quantity of the filtrate would give the same amount of protection. If a smaller amount of the filtrate was found to give a smaller protection then we must conclude that the ratio of antigen to nucleoprotein was not materially altered by the filtration. This, as the following experiments show, was found to be the case :

*Reports on Plague Investigations in India* 343

Dose	No. rats	No. survived	% survived
1/20 c.c.	23	13	56
1/200 c.c.	23	13	56

Reference to the previous reports (vol. X. (1910), p. 559 and vol. XI. (1911), supplement, p. 40) will show the amount of protection following the inoculation of a one-month's hydrolysed extract. Thus on p. 40 of the second report 53% survived after a vaccinating dose of 0.001 mg., an amount comparable with that present in the solution used above.

*We must conclude that filtration through gelatin has effected no separation of antigen from protein.*

## LVIII. BESREDKA'S METHOD OF VACCINATION.

By SYDNEY ROWLAND, M.A., M.R.C.S.

*Of the Lister Institute.*

A METHOD of vaccination that has achieved considerable reputation is that advocated by Besredka of the Pasteur Institute, Paris<sup>1</sup>. "Vaccination par les virus sensibilisés," as he terms his method, consists essentially in the use of killed cultures of the organism concerned. In this it does not differ, so far as concerns plague, from the method of Haffkine. But Besredka claims that, if the killed organisms are submitted to a preliminary soaking in a serum containing the specific antibodies to the organism concerned, these antibodies neutralise the toxic substances contained in the bacilli, which can then be used as a vaccine having the advantage of being atoxic.

The present position of the author of this method will be found described in the *Bulletin de l'Institut Pasteur* (1910), vol. VIII. p. 241, where he says "le vaccin antipesteux sensibilisé est depourvu de toute action toxique."

Now in the case of the plague bacillus we can extract the specific endotoxine (for rats), consequently we are in the position of being able to control this claim. Assuming that a given quantity of plague bacilli will normally yield a certain definite amount of endotoxine, then it is obvious that, if Besredka's explanation of the principles underlying his method be correct, the same quantity of plague bacilli will, after soaking in the antiserum, yield a smaller amount of endotoxine or none at all.

The following experiments were undertaken to determine

(1) The toxicity of a "whole" vaccine prepared according to Besredka's method.

(2) The amount of endotoxine that could be extracted from plague bacilli before and after soaking (sensitising) in antiserum.

<sup>1</sup> See the reviews by Besredka in *Bulletin de l'Institut Pasteur*, vol. VIII. (1910), p. 241 and vol. x. (1912), p. 529.



*Experiment I. Determination of the toxicity of a vaccine prepared according to Besredka's method.*

In this experiment the relative toxicity of three vaccines is determined. The three vaccines are comparable in every way except that in the case of vaccine 1, the bacilli were soaked (sensitised) in an anti-plague serum; in the case of vaccine 2 they were soaked in normal horse serum; and in the case of vaccine 3 they were soaked in physiological salt solution.

The serum employed in the case of vaccine 1 was prepared by immunising a horse by means of the endotoxine of the plague bacillus as already described (*Journal of Hygiene*, vol. XI. (1911), supplement, p. 11). At the time of the experiment this serum was of such a strength that one cubic centimetre neutralised 150 lethal doses of endotoxine. The proportion of serum to bacilli was so arranged that there was present in the quantity of serum employed sufficient antitoxin to neutralise four times the amount of endotoxine present in the bacilli.

These relations having been determined by preliminary experiments, 100 Roux bottles of agar were inoculated with plague and incubated for four days at 34° C. The bottles were then heated to 60° C. for an hour to kill the bacilli. This is the temperature and duration of heating employed by Besredka. The growth was washed off in salt solution (0·8%) and the bacilli twice washed in salt solution in the centrifuge. After the last washing the bacilli were again centrifuged and 41 grammes of paste obtained. This paste contained 13·84% of solids when dried at 105° C. Ten grammes of the paste were put in each of three flasks containing glass beads and there were added 35 c.c. of antiplague serum, normal serum and salt solution respectively. When a uniform distribution of the organisms throughout the respective fluids had been effected the flasks were put aside in a cool place till the following day. Meanwhile the quantity of bacilli in emulsion 3 was determined. It was found that the strength of this emulsion as determined directly corresponded with the strength as calculated from the analysis of the paste from which it was made. We may take it then that the paste of organisms was homogeneous, that the process of emulsification was adequate and that the three emulsions were comparable in the amount of organisms they contained.

The lethal dose of the three emulsions was then determined by subcutaneous inoculation into rats. Two rats were used for each dose :

Dose mg.	Emulsion 1	Emulsion 2	Emulsion 3
10	Died 2 days	Died 2 days	Died 2 days
	"	"	Survived
9	"	Survived	Died 2 days
	"	"	Survived
8	"	"	Died 2 days
	Survived	"	"
7	Died 3 days	"	"
	Survived	"	Survived
6	"	"	Died 2 days
	"	"	"
5	"	"	Survived
	"	"	"
4	"	"	"
	"	"	"
3	"	"	"
	"	"	"
2	"	"	"
	"	"	"
1	"	"	"
	"	"	"

The lethal dose of the three emulsions does not differ to any great extent. Outside the error of this kind of experiment there is no difference discoverable between the lethal dose of the organism that had been soaked (sensitised) in the antiplague serum and those that had been treated simply with salt solution. A slight apparent difference is noticed in favour of the organisms that had been sensitised in the normal horse serum.

A repetition of this experiment showed no diminution of toxicity in the case of the bacilli treated with serum.

*Experiment II. Estimation of the amount and toxicity of the endotoxine that can be extracted from plague bacilli before and after soaking in antiplague serum, normal horse serum and salt solution.*

A paste of organisms was prepared as in the last experiment: 32.5 grammes of paste were obtained containing 15.6% solids.

Three suspensions of the paste were made as in the last experiment.

Suspension 1	contained	10 gr.	paste in	37.5 c.c.	antitoxic serum.
"	2	"	"	"	normal horse serum.
"	3	"	"	"	salt solution.

The three suspensions were left in a cool place until the following morning. They were then centrifugalised and the paste of organisms washed free from serum proteins. The final washed paste obtained was mixed with twice its weight (in each case) of anhydrous sulphate of soda. The semi-fluid mass thus formed soon set to a solid mass which was reduced to powder. The full description of this process

of extracting the endotoxine of the plague bacillus has already been given in these reports (vol. x. (1910), p. 553). The powder was dissolved in warm distilled water and in such a volume as to form a saturated solution of sodium sulphate. After filtering off the solution the residue on the filter consisting of plague bacilli, sensitised or not as the case might be, was extracted with distilled water. The same amount of water was of course used in each case. Three extracts were thus obtained which contained the endotoxine from the three portions of bacilli which had been treated in the three ways indicated. Each extract was filtered through hardened filter paper to remove the bodies of the bacilli and the three filtrates examined as follows.

First the amount of nucleoprotein in each was determined by boiling a portion after acidification with acetic acid, drying and weighing the precipitate obtained. The weights of nucleoprotein in the three extracts in milligrammes per c.c. was as follows:

No. 1	No. 2	No. 3
6.62	6.34	6.66

These figures are very similar. There is no appreciable difference in the weight of endotoxine (nucleoprotein) extracted in the three cases.

Having thus failed to find any difference in the quantitative yield of nucleoprotein in the three cases, it remained to ascertain whether there was any qualitative difference in the three extracts. For this purpose the lethal doses of the three extracts were determined on rats. For the purposes of this determination the three extracts were made up to such a strength that 5 c.c. of each extract contained 1 milligramme of nucleoprotein. The three solutions were inoculated subcutaneously into rats as follows, two rats being used for each dose:

Dose mg.	Extract 1	Extract 2	Extract 3
1.0	Died 1 day	Died 1 day	Died 1 day
	"	"	"
0.8	"	"	"
	"	"	"
0.6	"	"	"
	"	"	"
0.4	Died 2 days	"	"
	Survived	Died 2 days	"
0.2	Died 1 day	Died 1 day	"
	Died 2 days	Survived	Died 3 days
0.1	Survived	Died 1 day	"
	"	Survived	Survived

The minimal lethal dose of the nucleoprotein obtained from bacilli that had been sensitised in immune horse serum was thus substantially

the same as the minimal lethal dose of the nucleoprotein obtained from the bacilli that had been treated (sensitised) in normal horse serum or in saline. The yield of nucleoprotein was also the same in the three cases.

From this experiment, which was repeated with similar results, it is concluded that on sensitising the plague bacillus in an antitoxic serum no permanent neutralisation of the endotoxine takes place.

The control experiment in which the bacilli were treated in salt solution shows that the effect of sensitising in either anti or normal serum is nil as regards the amount of nucleoprotein or the toxicity of the nucleoprotein.

I am therefore unable to confirm Besredka's statement that sensitised organisms yield an atoxic vaccine, for the whole organisms after sensitisation are just as toxic as before and no neutralisation of the endotoxine takes place as the result of the sensitising.

As the immune serum neutralised the endotoxin after extraction from the bacilli it must be concluded from these experiments that the antitoxine cannot enter the bodies of bacilli killed by heat.

There is, however, evidence that Besredka's vaccine is possessed of good immunising power and users of it have reported favourably as to the minimum of discomfort following its inoculation. A great point of this latter property is made by Besredka. Paladino-Blandini (*Annali d'Igiene sperimentale* (1905), pp. 295, 411), after an exhaustive examination of several methods of antityphoid vaccination, speaks in terms of high praise as to this property of Besredka's vaccine. Dopter (*Annales de l'Institut Pasteur* (1909), vol. XXIII. p. 677; *C. R. Soc. Biol.* vol. LXIV. (1907), p. 379), working with dysentery, speaks to the same purpose.

Besredka attributes the advantages of his sensitised vaccines firstly to an actual neutralisation of the endotoxin and secondly to sensitisation (opsonisation) of the bacteria. For the former I can find no support from my experiments with plague, but immune horse serum does contain opsonin. At one time Besredka recommended the use of normal horse serum in the preparation of his sensitised vaccine as being equally efficacious. Normal serum, however, can hardly be supposed to be antiendotoxic or possessed of any considerable specific opsonising power.

A further possibility is that after soaking in immune serum the bacilli are more readily lysed when placed under the skin. In this respect treatment with normal horse serum may be advantageous, for

whilst engaged in some investigations into the mechanism of plague immunity I found that in the normal horse a natural amboceptor for the plague bacillus exists in considerable quantity. It is possible that in Besredka's method this natural amboceptor is responsible for sensitising the bacilli so that they dissolve more rapidly after injection. Whilst at first Besredka employed specific sera for his sensitisation, later he abandoned these for the use of normal horse serum. Later still he reverted to the use of specific sera and makes the statement that it is necessary for these to be highly agglutinating.

The main experiments that led to the recognition of the normal amboceptor in the serum of normal horses are as follows:

(1) 80,000,000 virulent living bacilli were added to 1 c.c. of fresh normal horse serum. The serum was kept at 37° C. No multiplication took place and at the end of 24 hours the serum was sterile.

(2) 83,000,000 living virulent bacilli were added to 1 c.c. of the same serum as was used in the last experiment. The serum had been heated to 58° C. for one hour. Multiplication took place and followed the same curve when the numbers were plotted against time as the same number of bacilli inoculated into broth.

(3) 10,000,000 living virulent bacilli were added to one c.c. of the same serum that had been heated. At the same time 0.04 c.c. of fresh normal rat serum was added to the tube. No multiplication took place and at the end of four hours only half a million bacilli were recognised.

The growth and fate of living plague bacilli were traced in horse serum by the use of a method of direct microscopical observation. Specially constructed observation slides were used which, combined with a very perfect method of dark ground illumination, allowed of a record of the appearances of the living organisms and of their number being made as time advanced (see below, p. 362).

The further fact that so far as they have been examined all antiplague sera prepared by Yersin's or some equivalent method are strikingly deficient in specific plague antitoxine supports the explanation above given.

# LIX. THE RELATION OF PSEUDO-TUBERCLE TO PLAGUE AS EVIDENCED BY VACCINATION EXPERIMENTS.

By SYDNEY ROWLAND, M.A., M.R.C.S.

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THE resemblance of the bacillus of pseudo-tubercle of rodents to that of plague is very striking. The work of Galli Valerio<sup>1</sup>, Zlatogoroff<sup>2</sup>, and MacConkey<sup>3</sup> bears evidence of this. It has even been suggested that the pseudo-tubercle bacillus should be rechristened and be known as the pseudo-plague bacillus. There is no constant morphological or staining character which will enable us to distinguish the one from the other, nor do we receive much assistance towards the attainment of this end from a study of the cultural and biological properties of the two organisms<sup>4</sup>. The differences are merely differences of degree. It is the same with regard to the agglutination, precipitin and, as far as they are known, complement deviation reactions (J. Henderson Smith<sup>5</sup>). The post-mortem appearances in both diseases may be so strikingly similar that one can do no more than hazard a guess as to which bacillus is the cause of them. The similarity in the appearances of a guinea-pig that has died of pseudo-tubercle to those of a guinea-pig that has died of plague is so great as often to amount to identity. The only well-defined difference between the two organisms is one of virulence, for whereas the rat is hardly, if at all, susceptible to pseudo-tubercle it is very susceptible to plague. The guinea-pig is usually regarded as being equally susceptible to either.

These great resemblances led Zlatogoroff and MacConkey to test the question of the possibility of immunising rats and guinea-pigs against plague by means of the pseudo-tubercle bacillus. Zlatogoroff failed to obtain cross-immunisation but MacConkey concluded that it

<sup>1</sup> *Cent. f. Bakt. Orig.* vol. xxxiii. (1903), p. 321.      <sup>2</sup> *Ibid.* vol. xxxvii. (1904), p. 513.

<sup>3</sup> *Journal of Hygiene*, vol. viii. (1908), p. 335.

<sup>4</sup> MacConkey, *Journal of Hygiene*, vol. v. (1905), p. 350 ; see also *ibid.* vol. viii. (1908), p. 305.

<sup>5</sup> Unpublished observations.

was possible to immunise both rats and guinea-pigs against plague by means of inoculation of cultures of the pseudo-tubercle bacillus. In the case of rats he employed a living culture as his vaccine, whilst he found that with guinea-pigs either a living or a filtered autolysed extract was efficacious. His conclusions being drawn from only a relatively small number of animals it was considered of importance that they should be confirmed or refuted, and if found to be correct that the scope of the enquiry should be extended so as to obtain amongst other things some estimate of the extent of the immunity produced.

*The immunity to plague conferred on guinea-pigs by pseudo-tubercle.*

A vaccine was prepared from a culture of pseudo-tubercle that came originally from Professor Pfeiffer.

*Preparation of the vaccine.* Twelve Roux flasks were inoculated with this culture and incubated for three days at 35° C. Five c.c. of chloroform were then introduced into each flask and the flasks were again incubated for four hours. At the end of this time 10 c.c. of salt solution were introduced into each flask and the growth emulsified. To 26 c.c. of this emulsion were added 134 c.c. of salt solution. This constituted the vaccine. Each guinea-pig received 0.5 c.c. of this vaccine subcutaneously and after an interval of 15 days a second dose of 1.0 c.c. It was reckoned that the first dose corresponded to  $\frac{1}{8}$ th agar tube and that the second dose corresponded to  $\frac{1}{4}$ th agar tube of average growth.

A month after the second vaccination each pig received  $\frac{1}{10}$  c.c. of a broth culture of living virulent plague subcutaneously. At the same time 10 control pigs received a similar dose.

There were 44 guinea-pigs vaccinated and not a single one died of plague or was noticed to be even ill, whereas nine out of the ten control pigs died of plague. The vaccination has, therefore, given rise to complete protection.

*The immunity to plague conferred on rats by pseudo-tubercle.*

MacConkey obtained indications of some degree of resistance to plague in rats which had previously been inoculated with living pseudo-tubercle bacilli. Of thirteen rats which were so treated five died of plague when tested five months after the last immunising injection. Of his five controls all died.

Accordingly an experiment was made on rats using the same vaccine and the same method of immunisation as in the case of guinea-pigs. Thirty-seven rats were used for the experiment, but no evidence was obtained that any protective effect was produced when tested with living virulent plague four weeks after the second dose of vaccine.

The difference in the reaction of the two classes of animals to the vaccination appears all the more remarkable when we remember the facility with which rats can be immunised against plague by previous treatment with dead plague cultures and the difficulty with which by this means guinea-pigs are rendered immune to the same disease.

*The immunity to plague conferred by the nucleoprotein of the pseudo-tubercle bacillus on rats and guinea-pigs.*

As in the case of the plague bacillus a water-soluble nucleoprotein can be extracted from the bacillus of pseudo-tubercle. The same methods which have already been described in these reports (this *Journal*, vol. X. (1910), p. 553) were applied to the pseudo-tubercle bacillus and an extract prepared similar in its chemical characteristics to that obtained from the plague bacillus. Thus, to give an example of one preparation, 19 Roux bottles were inoculated and after four days' incubation at 34° C. a good growth was obtained. This was swept off in salt solution and the bacilli centrifuged down. The paste of organisms was emulsified in salt solution and again centrifuged in order to wash the bacilli. This process was repeated again. No chloroform was used in the process. To the final washed paste an equal weight of anhydrous sulphate of soda was added. The semi-liquid mass thus produced was rubbed in a mortar until on cooling it became a dry powder. The powder was dissolved in twice its weight of warm distilled water. A solution of sulphate of soda is obtained by this means which is of such a strength as to completely precipitate all the bacterial proteins. This solution was filtered off and the residue of organisms suspended in distilled water to extract. After an hour the extract was filtered through hardened paper and a clear fluid obtained. This clear fluid was found to contain 10·8 mg. of nucleoprotein per c.c. Unlike the extract obtained from the plague bacillus by the same treatment it was without toxicity for either rats or guinea-pigs. Guinea-pigs and rats survived doses of 10 mg.

Numerous experiments were made to determine whether this extract was possessed of any immunising value against living plague but all with a negative result. Thus, in one series of 26 rats the mortality



of the vaccinated animals was 18 % greater than the mortality of the controls and in another series of 26 guinea-pigs it was only 6 % less. In both these series the vaccinating dose of the nucleoprotein was 0.01 mg. and the test dose of plague was given two weeks after the vaccination.

Further experiments were made under the same conditions of testing as have been used in determining the immunity against plague following the inoculation of various vaccines, the technique of which is detailed in the first of these reports. The results may be tabulated as follows:

	Dose	No. animals	Result	% mortality
Rats	0.1 mg.	23	12 died of plague	52
	0.01 "	5	5 " "	100
Guinea-pigs	0.1 "	22	13 " "	59
	0.01 "	28	19 " "	68
Controls not vaccinated:				
Rats		19	14 " "	73
Guinea-pigs		15	9 " "	60

In the case of rats which received the 0.1 mg. dose a slight immunity is noticed.

In the case of the guinea-pigs no immunity is evident.

We must conclude that by the method employed the substance contained in the pseudo-tubercle bacillus which immunises guinea-pigs against plague cannot be extracted.

If it cannot be extracted and if it is not destroyed by the process used in attempting to extract it, it must be insoluble in dilute saline and remain in the bodies of the bacilli after the extraction. No experimental determination of this question has yet been made, but assuming that it remains in the bodies of the bacilli it presents a striking contrast to the antigen of plague which is effective in the case of rats.

The plague antigen for rats is easily extracted from the plague bacillus, which bacillus under similar treatment yields little or no antigen for guinea-pigs. A plague antigen for guinea-pigs is contained in the pseudo-tubercle bacillus from which by the method employed it cannot be extracted. The antigens for the two animals are, therefore, different.

The mechanism of immunity in the two classes of animals is therefore different—a fact of great importance in the art of vaccination. And it therefore behoves us to be careful in applying the results obtained from the study of a particular species of animal to man.

*Note on the toxicity of the plague nucleoprotein for guinea-pigs.* The nucleoprotein obtained from the plague bacillus and which, as has been shown, is invariably toxic and possessed of antigenic powers for rats is almost without action on guinea-pigs.

This conclusion is the result of repeated trials. The following test of a sample that was prepared without chloroform in order to eliminate the possible neutralising action of this substance may be given:

Dose	Rats	Result	Dose	Guinea-pigs	Result
5.4 mg.	1	Died 3 hours	27 mg.	1	Survived
	2	Died 1 day		2	"
2.7 "	3	"	18 "	3	"
	4	"		4	"
1.3 "	5	"	2.7 "	5	"
	6	"		6	"
0.1 "	7	"	1.3 "	7	"
	8	"		8	"

Coincident with this absence of toxicity, there is a minimum amount of protective power as the following table shows:

No. guinea-pigs	Protecting dose	Result	% survived
20	11 mg.	14 died	30
20	5 "	17 died	15

Controls received living plague only:

40	—	37 died	7
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The slight balance in favour of the vaccinated animals is not significant in view of the fact that pseudo-tubercle is a common disease amongst laboratory guinea-pigs and the knowledge we now have as to the protective value of this bacillus against plague.

### *Summary.*

It will be as well to summarise the above facts:

1. *The plague bacillus is equally virulent for guinea-pigs and rats, but whereas it is easy by either the whole bacillus or the contained nucleoprotein soluble in saline to immunise the rat against plague it is almost impossible to immunise the guinea-pig, either by means of the bacillus or its nucleoprotein.*
2. *The pseudo-tubercle bacillus is almost without pathogenicity for rats whereas the guinea-pig is susceptible to it.*
3. *It is easy to immunise the guinea-pig against plague by means of the pseudo-tubercle bacillus; very difficult to immunise the rat.*

4. *It was not found possible to immunise either the rat or the guinea-pig against plague by means of the nucleoprotein contained in the pseudo-tubercle bacillus.*

5. *Whereas the nucleoprotein of the plague bacillus grown on broth agar is toxic for rats it is hardly so at all for guinea-pigs. The nucleoprotein obtained from the pseudo-tubercle bacillus is relatively innocuous to either rats or guinea-pigs.*

These facts suggest that for a bacillus or bacillary product to act as an efficient vaccine it must in its original condition be possessed of toxic action for the animal to be protected. Thus the plague bacillus is toxic for rats and can be used as a vaccine, the nucleoprotein obtained from it is toxic for rats and will protect them, but it is not toxic for guinea-pigs and will not protect them. The pseudo-tubercle bacillus is pathogenic for guinea-pigs and will protect them, the nucleoprotein obtained from it is toxic to neither rats nor guinea-pigs and is incapable of protecting either.

This statement appears at first sight as opposed to the facts established in a previous report as to the independence of the toxic and antigenic properties of the nucleoprotein obtained from the plague bacillus, but this is not so. The mother substance from which the efficient vaccine is to be obtained must be of a toxic nature towards the animal it is desired to protect, but it is possible by hydrolysis to abate or destroy its toxicity without interfering with its antigenic properties. It would seem as if the antigenic and toxic properties of the nucleoprotein were closely connected—possibly a common molecular grouping is an essential part of both.

In this connection we are reminded of the established relations that exist in the case of diphtheria between toxins and toxoids. Diphtheria toxin on keeping spontaneously degenerates into toxoids. The endotoxine of the plague bacillus does the same, that is if we admit that the atoxic product of the hydrolysis of the toxin is analogous to the toxoid of diphtheria. These analogies are sufficiently apparent, but it should be noted that whereas in the case of plague the toxoid—so to name it—produces in animals not only antitoxines but antibacterial bodies as well, in the case of diphtheria the toxoid, and for that matter the toxin itself, produces only antitoxic substances.

*An apparent conversion of a virulent plague bacillus into the bacillus of pseudo-tubercle.*

These experiments and the conclusions based thereupon emphasise the close connection existing between the bacillus of plague and that

of pseudo-tubercle. The only test capable of distinguishing between the two organisms is an inoculation test. Pseudo-tubercle will not kill rats with the signs and symptoms of plague. To this may perhaps now be added that the nucleoprotein obtained from the pseudo-tubercle bacillus will not immunise rats against plague. But if the animal test fails us, how are we to distinguish between the two organisms? In that case it would appear that there is no means of settling the question. If two organisms from different sources cannot be distinguished by any known test it must for the time being be assumed that the two organisms are identical. If then we can so manipulate the bacillus of plague as to make it conform in its behaviour towards animals in all respects with that of the bacillus of pseudo-tubercle, then we must conclude that the two organisms are fundamentally the same, and relegate the difference in their effect on animals to the category of virulence.

We now proceed to show that by appropriate treatment the differences between a plague organism and the bacillus of pseudo-tubercle as regards their effect on animals (rats and guinea-pigs) can be made to disappear.

Some years' experience in the maintenance of a virulent strain of plague at its full virulence over a long period of subculture has suggested the following procedure. The culture is kept under such conditions as reduce the rate of growth to a minimum, and when it is required for test purposes it is put under such conditions as make the rate of growth a maximum. This amounts to keeping the stock culture cool and immediately before use transferring it to fresh medium at the optimum growing temperature. Any conditions which allow the bacilli to grow in the presence of their own products seem to lower the virulence and *vice versa*.

In conformity with this experience the experiment was tried of growing the stock virulent plague culture in a solution of the nucleoprotein obtained from the plague bacillus itself. Good growth took place. After two days' growth the culture was inoculated into a series of rats and guinea-pigs with the following results:

Ten animals were used in each series. Among the ten rats no acute deaths took place. One died on the fifth day with no signs of plague, three others died on the second, eighth and tenth days respectively. In none of them was there any signs of plague. Of ten control rats inoculated at the same time with the same culture grown in ordinary broth seven died of acute typical plague.

Growing the virulent bacillus in its own nucleoprotein has thus

abolished the virulence of the organism as regards rats. The dose of the cultures (0.1 c.c.) administered was the same in both cases<sup>1</sup>.

It would seem then that the effect on rats of the inoculation of a culture grown in a solution of its own nucleoprotein is the same as would be produced by the inoculation of a typical culture of pseudo-tubercle, the usual results of such an inoculation being the death of a certain number of the rats without any noteworthy signs. From such inoculated rats it is impossible to recover the pseudo-tubercle bacillus<sup>2</sup>.

Now as to the effect on guinea-pigs of the same culture.

Ten guinea-pigs were inoculated with the same dose as the ten rats. Of these ten pigs all died. The days on which they died were the eleventh (three), fifteenth and seventeenth respectively. On the fifteenth day five pigs were so ill that they were killed. All were examined post-mortem. In all the appearances were the same, viz. those typical of pseudo-tubercle. In this opinion Dr MacConkey, who examined the animals, quite agreed.

Ten control guinea-pigs inoculated with the same culture grown in ordinary broth gave the following results: nine died, the times of death being 6, 8, 6, 7, 6, 3, 5, 5, 5 days respectively.

By growing the virulent bacillus in the solution described it appears that not only is the day of death postponed, which is characteristic of pseudo-tubercle, but in addition the post-mortem appearances have changed from those typical of acute plague to those typical of pseudo-tubercle. This latter point must not be pressed too far as it is often impossible to distinguish between the two appearances. A known culture of plague will sometimes kill guinea-pigs with post-mortem signs indistinguishable from those of pseudo-tubercle.

The broad fact remains that in these experiments we have converted the bacillus of plague which kills rats acutely into a bacillus which will hardly kill them at all and then without any of the post-mortem signs of plague. On the other hand as regards guinea-pigs we have converted the acute killing bacillus into a chronic killing bacillus. In fact the bacillus that is found in the culture of the plague bacillus in the nucleoprotein solution is indistinguishable from a typical pseudo-tubercle bacillus.

<sup>1</sup> Even if the density of the cultures was not so nearly the same as it appeared to be, it has already been shown (vol. x. p. 543) that the fatality does not vary much with the dose within wide limits about  $\frac{1}{10}$  c.c.

<sup>2</sup> Personal communication by Dr MacConkey, and Dr Martin tells me he has had the same experience.

## LX. OBSERVATIONS ON THE MECHANISM OF PLAGUE IMMUNITY.

By SYDNEY ROWLAND, M.A., M.R.C.S.

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THE FATE OF THE PLAGUE BACILLUS AFTER INOCULATION INTO RATS.

### I. PERITONEAL INOCULATION.

#### 1. *The mechanism in normal animals.*

THE introduction of plague bacilli into the peritoneal cavity is followed by a reaction which is typically inflammatory. The first effect is a secretion of fluid containing cellular elements. The fluid is rich in a fibrin-forming mechanism, and the cells are so numerous as to give the fluid a milky appearance. They are during the first hours principally mononuclears and lymphocytes, the polynuclears appearing in force about the third hour. The cells are actively phagocytic but not uniformly so. It is common to find amongst a mass of polynuclears a minority that are crowded to bursting point with bacilli and a majority that are quite free from bacilli. Examined microscopically (on the warm stage) both are found to be actively amoeboid so that there is no question of killing by, for example, endotoxine. The fact is difficult of explanation.

The fluid, as has been remarked, readily clots. This clotting power forms the basis of a mechanism of great beauty. If a little of the exudate be removed and examined microscopically on the hot stage, delicate fibrils of fibrin are seen to form and to traverse the field in all directions. To these fibrils the bacilli become attached. So marked is this effect that the first evidence of the formation of the fibrils is often the arrangement of the bacilli in lines. A network of fibrils is thus formed traversing the fluid and enclosing in its meshes bacilli and cells. Soon the network, or more aptly the spongework, as it is extensive in all dimensions, begins to contract. The contraction continues until there results a mass of cells, bacilli and fibrin inextricably entangled.

In this way are formed in the peritoneal cavity small flecks floating in the exudate. Many of the cells are full to bursting point with organisms, others are plasmolysed setting free their granules, a few appear healthy. In some cases it appears as if so many cells have coalesced that the bacilli are lying in a syncytium of cells. Prominent in such a mass of cells are the eosinophile granules and the large basophil granules characteristic of the rat. The whole process is, in its first effects, a very perfect netting mechanism, resulting in a close aggregation of cells and bacilli. The effect is to clear the peritoneal cavity of bacilli and to bring them into intimate association with inflammatory cells. In this process the omentum plays a part. The ultimate fate of the flecks as I have called them above is to become attached to the omentum. If sufficiently numerous the omentum becomes retracted and rolled up enclosing masses of the flecks. In time, if the animal survives, the centre of the mass may become an abscess cavity.

In the course of the above process the animal may die at any stage. This death is presumably due to the absorption of endotoxine, for invasion of the body by bacilli has not yet taken place. At any time during the process there are observed bacilli which are free and have escaped the netting, and the fate of the animal depends on the completeness with which the process can accomplish itself. On the one hand we have the process as described progressing in an orderly sequence, on the other we have the bacilli multiplying at a certain rate. The fate of the animal depends on these two rates. If the rate of the process can compete successfully with the rate of multiplication then the animal survives, if on the other hand the rate of multiplication of the bacilli is greater than the mechanisms of netting, lysis and phagocytosis can compete with, then the animal succumbs to plague.

## 2. *The mechanism in immune animals.*

In an immune animal (*i.e.* an animal that has been vaccinated so that out of 100 animals so vaccinated 80 survive a certain lethal dose of virulent plague) precisely the same course of events takes place. At any stage the differences observed are not differences of kind but of degree. Thus whereas in the normal animal the number of bacilli escaping the process are many, in an immune animal there will be no bacilli outside the net. The extent of the reaction in the normal and immune animals seems to be the same and to depend more on the quantity of bacilli present than on anything else. *The number of bacilli on the other hand in the case of the immune animal is at any stage of the*

*process much less than is the number at the same stage in the case of the normal, unvaccinated animal.*

The differences observed in the two cases are all explicable on the assumption that in the case of the immune animal the multiplication of the bacilli is much less than in the case of the normal animal.

## II. SUBCUTANEOUS INOCULATION.

As peritoneal infection is hardly a natural mode of infection, it was possible that the conclusions drawn from its study might be erroneous. The usual method of infection in plague is a cutaneous or a subcutaneous inoculation, both in the laboratory or in the natural epidemic. A series of rats were accordingly inoculated subcutaneously with virulent and avirulent plague. A method had to be devised for following the course of events. It was possible to cut series of sections and to study them at leisure. A consideration of the difficulty and labour of getting a comprehensive view of the course of events from some thousands of sections led to its abandonment and the following method was employed. A rat was inoculated subcutaneously, taking care that the dose was deposited close under the skin, so that it might be possible to recover the bacilli and exuded cells from the point of inoculation, and to examine the condition of affairs by the method of smears. This method has the advantage that a living preparation may be put up and examined on the hot stage at any time. It is essential that the bacilli should be deposited in a limited area which can be subsequently identified. This is best ensured by inserting the needle from the opposite side of a limb until its point can be felt pushing up the skin on the other side. About  $\frac{1}{10}$  c.c. of culture is then deposited close under the skin at the point selected. The needle is then withdrawn through the muscle of the limb.

When it is desired to examine the course of events the whole skin is reflected including the subcutaneous tissue. The point at which the bacilli were deposited is then recognised through the fat and areolar tissue as a whitish spot. This is opened and smear preparations made and stained, usually with Leishman's stain. Apart from the usual and well known inflammatory reaction which is common to the presence of any foreign body the following specially interesting points were observed.

The difference between the reaction in the case of the normal and the immune rat was, as in the case of the peritoneum, one of degree and not of kind. Whereas in both cases the bacilli increased, in the



case of the immune rat the number of bacilli recoverable at any stage of the process was always much less. Concurrently the extent of the reaction in the immune rat is always much less than in the case of the normal rat, the extent of the reaction depending, other things being equal, on the number of bacilli present.

The conclusion reached was that the rate of increase of the bacilli in the normal rat was always much greater than was the rate of increase in the case of the immune rat. This conclusion may be expressed in another way by saying that the normal rat provides a good culture medium for the plague bacillus whereas the immune rat provides a very bad one.

One series of rats examined in this way may be especially alluded to. It was a series inoculated with a strain of plague that had become, on long subculture in the laboratory, almost completely avirulent. The reaction in this case was of the same typical inflammatory character as in the case of the virulent bacillus. Day by day it was possible to examine the condition of affairs for a week. This was possible as the bacillus being avirulent the rats did not die. For as long as four days after the inoculation free bacilli were found at the site of inoculation. A certain amount of phagocytosis had taken place but this was not complete, bacilli were still after this long time slowly multiplying, unattacked by the phagocytic process. Under the same circumstances of inoculation but with a virulent bacillus the rat would in all probability have been dead by the end of the fourth day. There would have been found at the site of inoculation a vast number of bacilli with a correspondingly extensive reaction. This reaction would not have differed in kind from the small reaction observed in the case of the avirulent bacillus. The difference in the two cases impressed on one is that the virulent bacillus can multiply readily in the body of the rat whereas the avirulent organism can only do so exceedingly slowly or not at all.

In the same way a virulent organism multiplies slowly in the body of a vaccinated rat and rapidly in the body of a normal rat. As regards the virulent organism in the body of the rat the effect of vaccination has been to convert the picture into that of an avirulent one in a normal rat.

It is impossible to give a quantitative measure of these experiments but from the examination of a large number of animals the conclusion is forced upon me that *the essential factor in plague immunity is one which affects the multiplication of the bacillus.*

## THE MULTIPLICATION OF BACILLI IN RAT SERUM.

We must now examine this question specifically and determine whether there is a difference in the rates of multiplication of plague bacilli in the body fluids of normal and immune rats. It will be convenient to begin with the serum.

*Method of investigation.* A method is required that will give at any moment the bacilli existing in the serum under examination. Plating was out of the question as it was soon found that the bacilli grew in chains when the medium contained serum, and one could not by plating discriminate between one bacillus and several hundred bacilli under these circumstances. The same applies to a dilution and end point method. The only method available is direct counting under the microscope. This involves the examination of a given volume spread in a layer of uniform thickness and an illumination capable of defining the individual elements distinctly. The first method tried was to use a Thoma counting cell as in the enumeration of blood elements and dark ground illumination with a paraboloid condenser. A difficulty arose owing to the fact that in serum the organisms settled very slowly; under these circumstances it was necessary to count throughout the depth of the cell and when the focus was adjusted to any one point save the bottom, on which the cross lines are engraved, the diffraction rings from these lines obscured the images of the bacilli. In addition the obliquity of the illuminating cone in the case of the paraboloid is so excessive that if the medium was not optically clear the diffracted images of the suspended particles further obscured the images of the bacilli. These difficulties were overcome by having special counting chambers made (by Zeiss) without any rulings. The place of the rulings was taken by two spider lines in the eyepiece. These are adjustable and can be set at any desired interval. They always remain in focus and the entire depth of the cell can be examined and the bacilli at all levels counted.

The difficulty of the obliquity of the illumination was overcome by discarding the paraboloid and using in its place the aplanatic condenser of Zeiss to which was adapted a suitable central stop. Most of the counts were made with the 8 mm. apochromatic objective of Zeiss with a central aperture stop. Some of the counts were made with the 4 mm. of the same series.

The dark ground effect with this arrangement is very perfect. The

bacilli stand out as bright rings on a dark ground and no difficulty arises from impurities in the medium, at least these difficulties are minimised. The depth of the cell is constant and known, the width is read off from the screw adjusting the distance apart of the spider lines in the eyepiece, the length is measured by a vernier attached to the mechanical stage. Thus the volume counted over is accurately known, and the number of organisms per unit volume can be expressed in any convenient terms. In what follows the units are millions per cubic centimetre.

If a small number of plague bacilli that have been grown on agar be introduced into fresh normal rat serum, growth as a rule fails to occur. If the fate of the introduced bacilli be watched hour by hour they are seen to gradually lose their bright outline (bright under the conditions of illumination employed) and to finally become indistinguishable. At the same time they lose their power of being stained. If a larger number be introduced the same fate befalls a proportion of them, the remainder after the lapse of some hours beginning to grow in chains. The proportion that begin to grow after surviving the lytic power of the serum is surprisingly small. Thus of 20 million organisms that were placed in fresh normal rat serum, only half a million were found after 10 hours' incubation at 37° C. The recognition of the living organisms under these circumstances is facilitated by counting the number of multiplying units. Thus in the case mentioned 20 million individual organisms were introduced, the bacilli being obtained from the surface of agar and emulsified in salt solution by the aid of shaking with beads until no clumps remained. After 10 hours, multiplication of the survivors in the serum had commenced and small chains of three or four organisms were seen. A count of the number of these chains gives the number of the individuals that survived the lysis in the normal serum. This lytic effect of normal serum is an obstacle in investigating the growth of plague in immune serum.

As we have seen, the plague bacillus contains within its body an endotoxine. On the occurrence of lysis this endotoxine is liberated and if an immune serum is under investigation the liberated endotoxine will neutralise the antibodies present in the serum. Thus the very substances whose presence it is desired to investigate become wholly or partially neutralised at the outset and the maintenance of a clean experiment becomes impossible.

This lytic effect of normal serum is not confined to plague; in

typhoid, for example, it constitutes a grave source of inaccuracy in the opsonic technique. The difficulty in this case has been overcome by the introduction of a dilution method. The adoption of such a method in the investigations with plague was not considered advisable as it was desired to investigate the growth of the organism under conditions as closely approximating to those obtaining in the body as possible. Another way out of the difficulty suggested itself. From one point of view the effects of the preliminary lysis may be regarded as breeding out from the agar or broth strain of organisms a serum-resistant race. This point of view is rendered probable from the consideration of some facts as to the numbers of bacilli lysed according to the number introduced into the serum. If the lytic process were one concerned with specific antibodies these would be used up in the process and the introduction of a sufficiently large number of organisms would exhaust them. This is not found to be the case, the number lysed being approximately a function of the number introduced. There is thus a selection among the bacilli, some of them being capable of growing in serum and others not.

On the other hand this lysis in normal serum appears to depend on the presence of fresh complement as it can be abolished by a preliminary heating of the serum. Eight million organisms were introduced into one cubic centimetre of rat serum that had been heated to 55° C. for half an hour, multiplication commenced and at the end of four hours the number had increased to 30 millions. In a parallel experiment with the addition of 0.02 c.c. of fresh rat serum there was no multiplication at the end of four hours.

A method of circumventing the complicating process of lysis in fresh normal serum is as follows. If a certain number of bacilli be introduced into fresh normal serum the survivors begin to multiply in chains which can be recognised at the end of say 10 hours as being of a certain length or composed of a certain number of organisms. The rate of multiplication of the survivors, as indicated by the length of the chains after the expiry of a certain time, is accelerated by the presence of endotoxine. Thus, in a certain experiment 20 million organisms were added to one cubic centimetre of fresh normal rat serum, and at the end of 10 hours the number was reduced to half a million and the survivors had not begun to multiply. In parallel experiments in which varying amounts of endotoxine had been added to a series of tubes containing the same amount of serum and bacilli the results were as follows:

Amount of endotoxine	Appearance of survivors
0 mg.	No chains
0·02 „	No chains
0·04 „	No chains
0·08 „	Chains of four or six
0·16 „	Very long chains

A preliminary lysis occurs, after which the rate of growth of the survivors is accelerated by the presence of the endotoxine. The fact that the endotoxine can be added in increasing amount before this effect is noticeable, suggests that a certain proportion is combined with the serum before its accelerating effect becomes apparent. If this experiment be repeated with immune serum the same effect is observed with this important distinction that the amount of endotoxine that must be added to produce the accelerating effect is very much larger. Thus in one experiment with fresh immune rat serum to which 10 million organisms had been added at the expiry of 10 hours the following effects were observed:

Amount of endotoxine	Appearance of survivors
0 mg.	No organisms recognisable.
0·2 „	„ „ „
0·4 „	0·06 mill. multiplying elements in short chains.
0·8 „	0·1 mill. elements in short chains.
1·6 „	0·2 mill. elements in long chains.
3·2 „	0·7 mill. elements in very long chains.
6·4 „	Luxuriant growth in chains and masses too numerous to count.

The appearance in the tube that had received 0·8 mg. in the case of the immune serum was similar to that in the tube that had received 0·08 in the case of the normal serum. These experiments were repeated with similar results. It would appear that the endotoxine of the plague bacillus prepares the serum in some way, rendering it a good culture medium for the organism and that this effect is neutralised by immune serum.

These experiments are incomplete. So far as they have been carried however they give some indication of the mechanism of the immunity conferred by the inoculation of the endotoxine.

The characters of this immunity as evidenced in the suggestive experiment last mentioned give ground for regarding it as an anti-endotoxic immunity. On this view the function of the endotoxine of the plague bacillus is to counteract the disadvantageous effect of some constituent of the serum and to render the rat a good culture medium for the bacillus. How it accomplishes this is not clear; it may well

be that the proteolytic enzyme contained in the bacillus and always present in the endotoxine plays a part. If so it must have a very high velocity coefficient. In experiments that have been made to test the anti-endotoxic power of horse serum it apparently makes little difference whether the mixture of toxin and serum is allowed to stand for more or less time before inoculation into the test animal.

*Whatever the mechanism, the fact remains that the same toxine that is capable of provoking the formation of antitoxine in the horse is capable of neutralising it in the immune serum of this animal, while when it is injected into rats it provokes an immunity to infection with the living organism, the essential features of which seem to be resident in the serum of this animal, and to be in turn capable of being neutralised by the endotoxine.*

# LXI. THE ONSET AND DURATION OF THE IMMUNITY CONSEQUENT ON THE INOCULATION OF PLAGUE NUCLEOPROTEIN.

By SYDNEY ROWLAND, M.A., M.R.C.S.

*Of the Lister Institute.*

It has been shown in a previous report (vol. x. p. 559) that consequent on the inoculation of the nucleoprotein obtained from the plague bacillus an immunity is established to the subsequent inoculation of living plague bacilli in broth culture. Some indication of the extent of this immunity was obtained by comparing the mortality of the inoculated rats after the test inoculation with the mortality that occurred (78 %) in uninoculated rats. In all these investigations the time that was allowed to elapse between the protective inoculation and the test inoculation was 14 days. We have now to consider the influence of the length of time elapsing between the protective and test inoculations upon the protection.

It has also been shown that the extent of the protection consequent on the inoculation of the nucleoprotein is of the same order in the cases where this substance is employed as a vaccine either in its fresh toxic condition or in its autolysed and relatively atoxic condition (vol. XI. supplement, p. 38). These two conditions of the nucleoprotein will also be considered in relation to the onset of immunity.

## ONSET OF THE IMMUNITY.

### I. *Immunity conferred on rats by inoculation of the nucleoprotein immediately after, before or simultaneously with the test inoculation.*

#### A. Using fresh toxic nucleoprotein in 0.01 mg. doses:

of 10 rats inoculated	4 hours before the test dose	7 died 3 survived
" "	" at the same time as the test dose	6 died 4 survived
" "	" 5 hours after the test dose	7 died 3 survived
" "	18 " " "	" "

#### B. Using 2 months autolysed nucleoprotein in 0.01 mg. doses:—

of 10 rats inoculated	4 hours before the test dose	6 died 4 survived
" "	" at the same time as the test dose	8 died 2 survived
" "	" 5 hours after the test dose	8 died 2 survived
" "	18 " " "	" "

From these experiments the conclusion is justified that the inoculation of the nucleoprotein either in its toxic or atoxic condition is without effect when the time that elapses between its administration and the test dose is less than 18 hours.

II. *What is the earliest time after the inoculation of the nucleoprotein at which evidence of protection against living infection is noticeable?*

A. Using toxic nucleoprotein in 0.01 mg. doses, *i.e.*  $\frac{1}{10}$  lethal dose:

1 day after the inoculation	8 died	16 survived, <i>i.e.</i>	66 %	survived
2 days	2	18	88	„
3 „	0	17	100	„
4 „	2	17	89	„
14 „	2	17	89	„

B. Using the relatively atoxic nucleoprotein in 0.01 mg. doses, *i.e.*  $\frac{1}{100}$  lethal dose:

1 day after the inoculation	8 died	11 survived, <i>i.e.</i>	60 %	survived
2 days	7	11	72	„
3 „	3	14	88	„
4 „	4	15	84	„
14 „	2	12	83	„

The results of these experiments may be put in the form of a curve when their meaning becomes more evident.

From these curves the following conclusions are justified:

1. That after the inoculation of nucleoprotein in 0.01 mg. doses the development of the resulting immunity is very rapid, being distinctly evident 24 hours after vaccination<sup>1</sup>.

2. The highest point on the curve, *i.e.* the greatest immunity, occurs on the third day.

3. That a slightly better immunity is evident on the third day after the inoculation of the more toxic vaccine.

The conclusion that for the rapid production of immunity it is advantageous to employ a toxic vaccine is capable of control in the following way. In previous reports it was pointed out (vol. XI. supplement, p. 36) that the toxicity of the nucleoprotein was greater the shorter the time elapsing since its extraction from the bacillus, and that there was reason to suppose that as it existed within the body of the bacillus its toxicity was at a maximum. Consequently if there is any relation between the toxicity and the rapidity of onset of the immunity, the vaccination by the same dose of nucleoprotein wrapped up in the

<sup>1</sup> Working with Haffkine's prophylactic as an immunising dose, wild Indian *Mus rattus* and an emulsion of a plague rat's spleen as an infecting dose, Stevenson and Kāpadia (*Report of the Bombay Bacteriological Laboratory for 1911*, p. 26) found evidence of some protection as soon as 8½ hours after vaccination.



bodies of the bacilli should be followed by a still earlier development of the maximum immunity. Using such doses of whole bacilli killed by heat (about 0.1 mg.) as contained 0.01 mg. of nucleoprotein, the following results were obtained:

1 day after the inoculation	18 survived	1 died, i.e. 95 % survived
2 days	16	3 " " 84 "
3 "	14	3 " " 82 "

These results are plotted in figure 1.

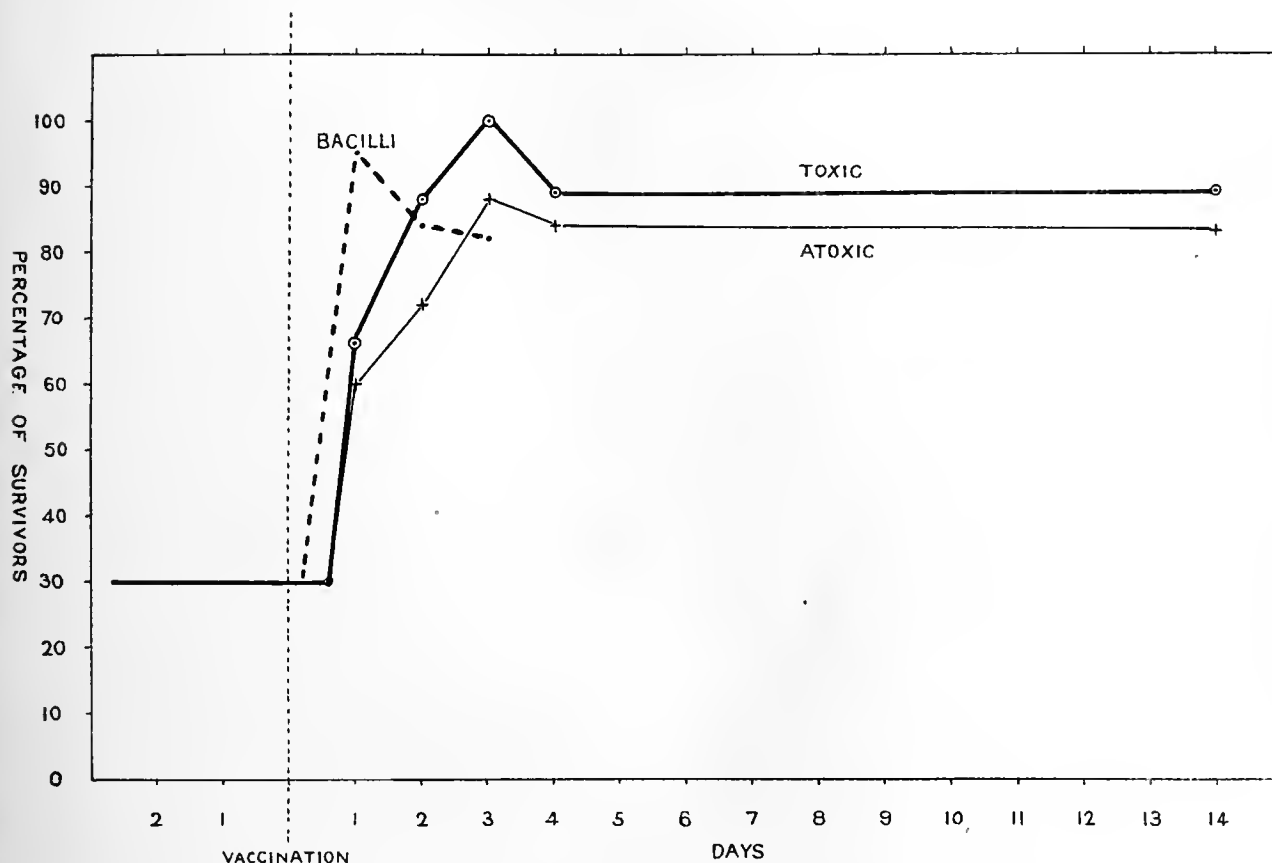


Fig. 1.

Considering the three curves together it will be seen that the highest point is reached first in the case of the whole bacillus vaccine, that is in the case of the vaccine in which we have reason to suppose that the contained nucleoprotein is in the most toxic condition. The conclusion was previously (vol. XI. supplement, p. 42) reached that the protective and toxic properties of the nucleoprotein contained in the plague bacillus were distinct. This conclusion was based on the consideration of the results of vaccination of rats when the interval between the vaccinating and the test dose was 14 days. At this time it remains true that there is no discoverable difference in the results of

the two (toxic and relatively atoxic) vaccines. But in the earlier stages of the onset of immunity a difference emerges. With the more toxic vaccine the onset of immunity is slightly more rapid; this early rise however appears to be associated with a corresponding early fall so that at the end of a fortnight there is no difference in the height of the immunity curve.

An observation of some interest was made in connection with the series of rats last quoted. These rats were inoculated (vaccinated) with whole bacilli that had been heated to 60° C. for half an hour. The resulting immunity was completely established as early as the first day. With a view to determining the fate of the bacilli used as the vaccine, certain of the rats were kept and the site of vaccination examined from day to day. There was no difficulty in recognising the presence of the bodies of these bacilli. Now it has been repeatedly shown that all the immunity that a culture of plague is capable of conferring on rats is represented in the nucleoprotein extract obtained from them by the methods described in these reports, the rest of the bacilli being incapable of conferring any immunity. In conformity with this, in the series under consideration the bodies of the bacilli were recognisable microscopically up to the fourth day after the inoculation, that is three days after the establishment of all the immunity that the vaccinating dose of organisms is capable of conferring. The resulting immunity must therefore have depended on the escape of the contents of the bacilli, the presence of any unabsorbed portion being hardly capable of being considered responsible.

#### THE DURATION OF THE IMMUNITY.

Already, on the fourth day after the vaccinating dose of nucleoprotein the differences observed in the height of the immunity curve according as fresh or autolysed nucleoprotein has been used for the vaccination are seen to be very small. After the expiration of 14 days these differences are no longer significant.

It is noteworthy however that the height of the curve remains constant after the slight drop between the third and fourth day, that is to say that the immunity once established remains at the same level. It will now be shown that this level is maintained for some months. The three curves being so nearly coincident at the expiry of 14 days the question of the differences in the extent of the immunity following the inoculation of the nucleoprotein in its fresh or autolysed condition will not be further considered.

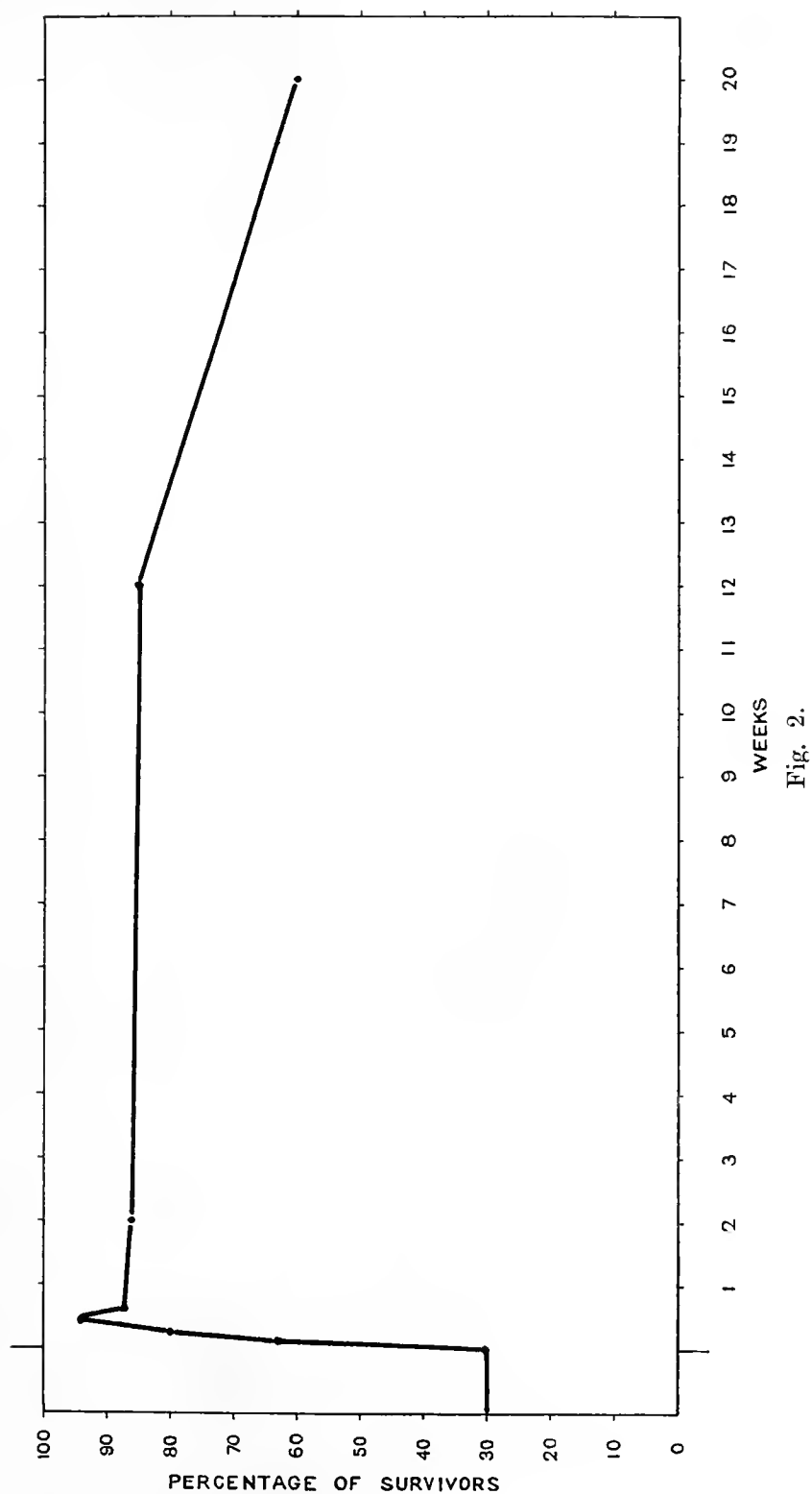


Fig. 2.

In connection with these experiments considerable difficulty has been encountered in keeping rats alive and healthy for long periods of time. It was hoped that the curve of immunity might be continued for a year at least. On two occasions some hundreds of rats were vaccinated and provision made for keeping them for a long period. This has not been found possible. Epidemics of various diseases broke out during the hot summer of 1911, and after the disappointing loss of much valuable material it has only been found possible to obtain reliable data up to five months after the vaccination (see fig. 2).

At three months after vaccination a series that received 0.1 mg. and one that received 0.01 mg. are available. Of the first series every one of 19 rats survived the test inoculation. Of the second 85 % survived, the series comprising 20 rats. The immunity thus lasts unimpaired for three months at the same height as it was at the end of 14 days.

At five months only one series of 31 rats was available. Of these 18 survived the test dose, *i.e.* 60 %, from which it would appear that in rats the immunity after a single dose of the nucleoprotein is still very appreciable after five months though a good deal less than after three months.

It need hardly be pointed out that the times ascertained in these experiments for the onset and duration of immunity in the rat cannot be directly transferred to any other species of animal, especially when the size and rate of metabolism are widely different from those obtaining in the rat.

## LXII. THE OPSONIC INDEX IN PLAGUE VACCINATION.

BY RALPH ST JOHN BROOKS, M.B., D.P.H.

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IN this paper an attempt is made to correlate the production of immunity in rats vaccinated against plague with the production of immune opsonin in the serum of such animals.

Various antibodies appear in the serum of animals inoculated with plague vaccines, but the agglutinins and bactericidal substances, for example, do not lend themselves to satisfactory quantitative study in the case of plague. The opsonic response has been studied in man by Douglas<sup>1</sup> who found a good response after inoculation with Haffkine's prophylactic. It, therefore, seemed advisable to correlate the development of opsonin with the absolute immunity of some susceptible animal in order to see whether the amount of opsonin produced could be used as evidence of the development of immunity in man.

In most of the following experiments the results obtained with a single dose of vaccine are recorded in relation to the opsonic index: in others the results of repeated dosage are shown. These results should be considered in the light of actual immunity experiments with test lethal doses of plague, as performed on vaccinated animals by S. Rowland and described above (p. 367).

The scope of the enquiry resolved itself into the following points:

1. Is there any correlation between immunity and opsonin-production in plague infection?
2. What is the influence of previous vaccination in the experimental animal?
3. What is the source of opsonin in immune plague serum?

*Methods of experiment.*

In the following series of experiments the dilution method of Klien (1907) was adopted.

<sup>1</sup> Communicated to the Pathological Society of London, 1906.

In high concentrations of immune serum phagocytosis of plague bacilli is obscured by (a) digestion of the bacilli inside the leucocytes, and (b) by clumping of bacilli. By means of the dilution method these difficulties are overcome. The ordinary method of incubating the opsonic mixtures in capillary pipettes did not give satisfactory results as the bacilli were found to precipitate and form clumps during the process, thus giving very uneven counts in smears from the same mixtures. I found the most satisfactory results were obtained by making the opsonic mixtures in small tubes which were subsequently placed in a shaking machine in a 37° C. incubator. The mixtures were incubated for a period of twenty minutes.

Throughout the period of experiment a strain of avirulent plague bacillus (K. 120) was used in the opsonic technique, and in vaccinations with the whole bacillus. The nucleoprotein of the plague bacillus used for vaccination was also prepared from the same source. The cultural characteristics of this bacillus have been given by Rowland (vol. x. p. 563).

The bacterial emulsion was prepared each day from a twenty-four hour agar slope, and throughout the period of experiment agar from the same batch was used. This fact is of some importance as it has been observed that great alterations in the phagocytic index may be caused by using bacilli grown on a fresh batch of agar during the time of experiment (Noon, 1909). The cultures were emulsified in 5% formalin solution and placed in the hot room (37° C.) for one hour in order to kill the bacilli. This was regarded as a necessary precaution on account of the danger in spreading slides containing live bacilli. In one case a control with live bacilli was made which showed that the employment of formalised cultures did not appreciably alter the phagocytic index. The ordinary methods of obtaining fine emulsions, such as shaking with glass beads, do not give good enough emulsions of plague bacilli for opsonic work. To get over this difficulty the following method was adopted. The killed formalin emulsions were decanted into small centrifuge tubes and spun down for a few minutes. The supernatant fluid was then poured off and the bacterial residue was thoroughly broken up by means of a small sable-hair brush, normal saline solution was added and the process repeated two or three times. Finally a short centrifuging was given to precipitate any remaining clumps, the top layers of the emulsion removed to a separate tube and then thoroughly mixed with a capillary pipette. Emulsions of approximately uniform turbidity were used throughout the experiments.

The pooled serum of ten rats was used for each experiment. The first bleeding was performed before inoculation. The rats were vaccinated subcutaneously in the thigh and were bled from the tails, a couple of drops of blood being taken from each animal. The blood was allowed to clot out over night at room temperature and the serum was separated each morning, and used for opsonic determination the same day. The serum dilutions were prepared immediately before the daily opsonic determinations.

Throughout the experiments I used preparations of my own leucocytes, prepared in the usual manner. The emulsions were used about one hour after preparation in order to give the leucocytes time to recover from any paralysis induced by centrifugalisation, but were not allowed to remain unused long enough to allow any deterioration of activity to set in. One hundred leucocytes were counted on each slide. Broken leucocytes and leucocytes in clumps of more than three were rejected in counting.

In each of the opsonic mixtures one volume of normal saline, one volume of serum dilution, one volume of bacterial emulsion and one volume of leucocytes were used. The counts of the falling dilutions were compared with a saline control in each case and controls of normal rat serum were from time to time employed<sup>1</sup>.

#### *Materials used as vaccine.*

The specific nucleoprotein employed was made by Dr Rowland in the manner described by him (Rowland, 1908). The powder "B" employed represents the bodies of the bacilli, with their contained nucleoprotein, in a mixture of anhydrous sodium sulphate. The number of grams of the powder used was added to twice the quantity of distilled water at 37° C. and the mixture was then filtered at 37° C. through a Büchner filter. The residue was scraped from the filter-paper and emulsified in distilled water and after centrifuging down a portion of the supernatant fluid was set aside for estimation of nucleoprotein. The nucleoprotein in this portion was precipitated by boiling after the addition of acetic acid and the precipitate carefully collected, washed, dried and weighed in the usual way. The amount of nucleoprotein in the original solution was thus controlled and the necessary dilutions made therefrom.

<sup>1</sup> With normal rat serum an opsonic effect was found in serum dilutions of  $\frac{1}{16}$  and  $\frac{1}{32}$  but never in higher dilutions than these.

When the whole bacillus was employed as a vaccine, the surface growth of six Roux bottles, incubated for five days and killed by heating for half an hour at 60° C., was swept off into 5 c.c. normal saline and thoroughly shaken up with glass beads in a stout flask. Three c.c. of this emulsion were then centrifuged down in a tared centrifuge tube and after several washings with distilled water, the residue was evaporated in a hot-air oven at 105° C. till the weight was constant. The remainder of the emulsion was then diluted down to the required strength. Although plague nucleoprotein is readily reduced in toxic and immunising properties by heating, Rowland has shown that this does not obtain so long as the nucleoprotein remains in the bodies of the bacilli (Rowland, 1911).

The slides containing the opsonic films were fixed with methyl alcohol and stained with dilute Giemsa stain for fifteen minutes.

#### A. EXPERIMENTS ON RATS.

##### *Experiment I.*

Each of a series of ten rats was inoculated with  $\frac{1}{100}$  mg. of freshly prepared nucleoprotein solution in 1 c.c. normal saline. The animals were bled before inoculation, ten hours later and subsequently every morning. The following table gives the results of opsonic counts from day to day.

	1	2	3	4	5	6	7
Serum dilution	Before inoculation	10 hours	22 hours	2 days	4 days	5 days	18 days
1/16	2.53	—	—	—	—	—	—
1/32	1.10	—	—	—	—	—	—
1/64	1.05	—	—	—	—	—	—
1/128	—	—	—	—	—	—	2.05
1/256	—	2.52	—	—	2.10	1.00	1.70
1/512	—	1.96	1.66	—	2.00	1.62	0.98
1/1024	—	1.80	1.58	1.50	1.02	1.49	0.91
1/2048	—	1.80	1.46	1.37	0.92	1.07	—
1/4096	—	1.00	1.73	.87	0.93	1.01	—
1/8192	—	0.98	1.10	.87	—	—	—
1/16384	—	—	1.02	—	—	—	—
1/32768	—	—	1.02	—	—	—	—
Normal saline control 1		1	1	1	1	1	1

Taking the highest dilution of serum which produces an effect greater than that of normal saline, the following result is obtained.

Before inoculation	10 hours	22 hours	2 days	4 days	5 days	18 days
1/16	1/2048	1/4096	1/2048	1/512	1/1024	1/256

The curve obtained by plotting out these figures is shown on chart I.



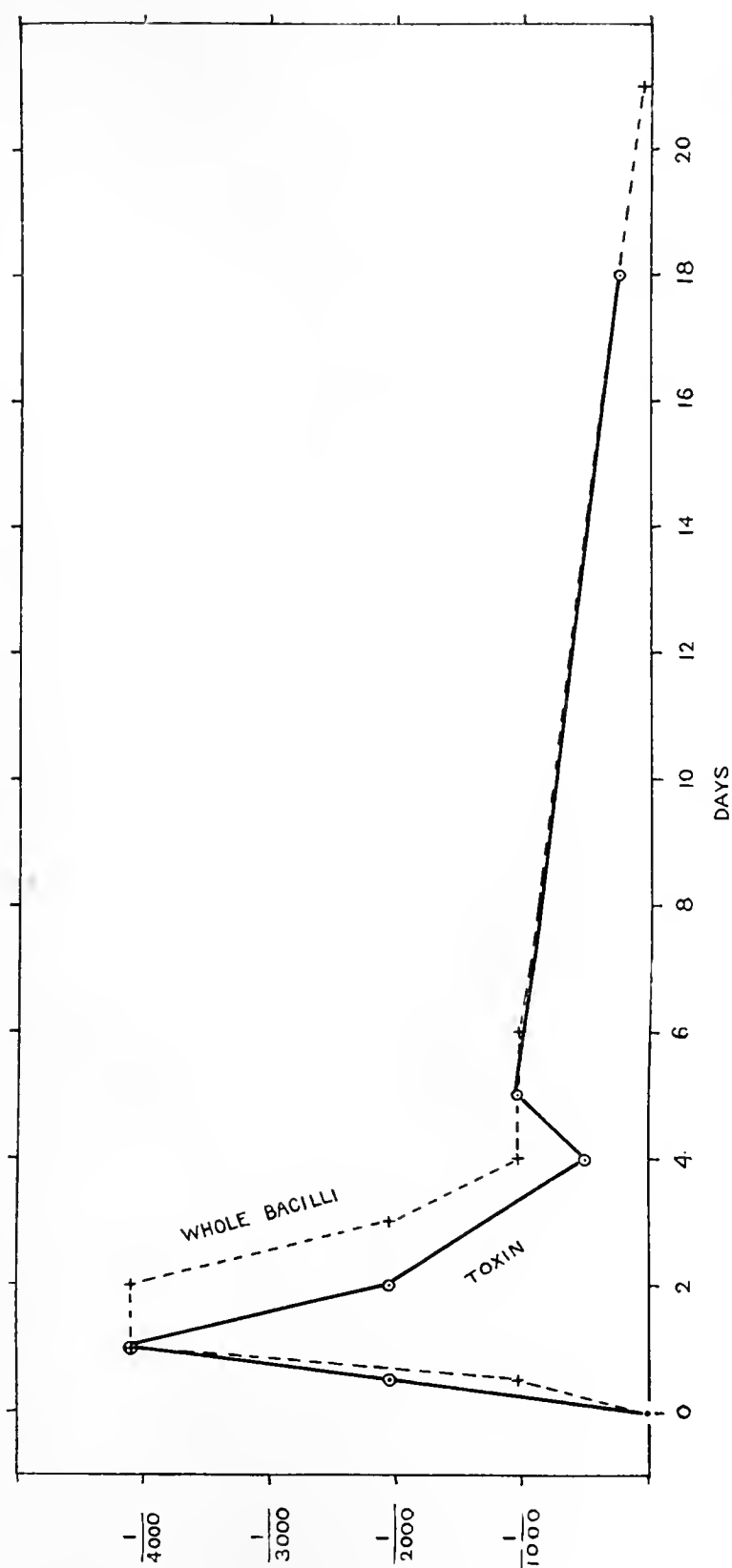


Fig. 1.

It will be seen from this that the maximum is obtained within 24 hours and afterwards the curve steadily falls so that on the eighteenth day a serum dilution of  $\frac{1}{512}$  corresponds to the saline control. It has been shown that full immunity to a lethal dose of virulent plague is obtained within the same period, thus showing that, for this dose of vaccine, the production of opsonin follows the immunity reaction, at least in the early period of immunity.

### *Experiment II.*

Ten rats were inoculated with  $\frac{1}{10}$  mg. of whole bacillus vaccine. Rowland has shown that the nucleoprotein content of the bacillus equals about  $\frac{1}{10}$  by weight of the whole bacillus, consequently this dose of whole vaccine is comparable to the dose of derived vaccine in experiment I.

The following results were obtained in this series, and the plotted curve obtained therefrom is also shown on chart I.

Before inoculation	10 hours	22 hours	2 days	3 days	4 days	6 days	21 days
1/16	1/1024	1/4096	1/4096	1/2048	1/1024	1/1024	1/64

The results obtained are similar to those obtained in experiment I. The optimum of the curve is displaced slightly towards the right. This difference is so slight that one would not be inclined to place too much significance on the fact, which, however, might possibly be due to the fact that the nucleoprotein in the second case takes an appreciable time to soak out of the bodies of the bacilli.

The general conclusions from these experiments would seem to show, as found by S. Rowland by directly determining the protection aroused in rats to a subsequent fatal dose of living plague, that the soluble nucleoprotein contained in the plague bacillus is the responsible agent in the production of opsonin.

### *Experiment III.*

Ten rats were each inoculated with a very small dose of nucleoprotein solution, *i.e.*  $\frac{1}{12000}$  mg. in 1 c.c. normal saline. At the end of ten hours samples of blood were taken but no opsonic response was observed. The curve commenced to rise the next morning and reached its maximum on the third day after which it gradually fell.

Before inoculation	10 hours	22 hours	2 days	3 days	4 days	5 days	6 days	8 days
—	—	1/512	1/1024	1/2048	1/2048	1/1024	1/828	1/512

Compared with the curves obtained with the larger doses in experiments I and II, it will be observed that with this extremely small quantity the maximum is postponed to the right and that the curve does not rise to anything like the same height.

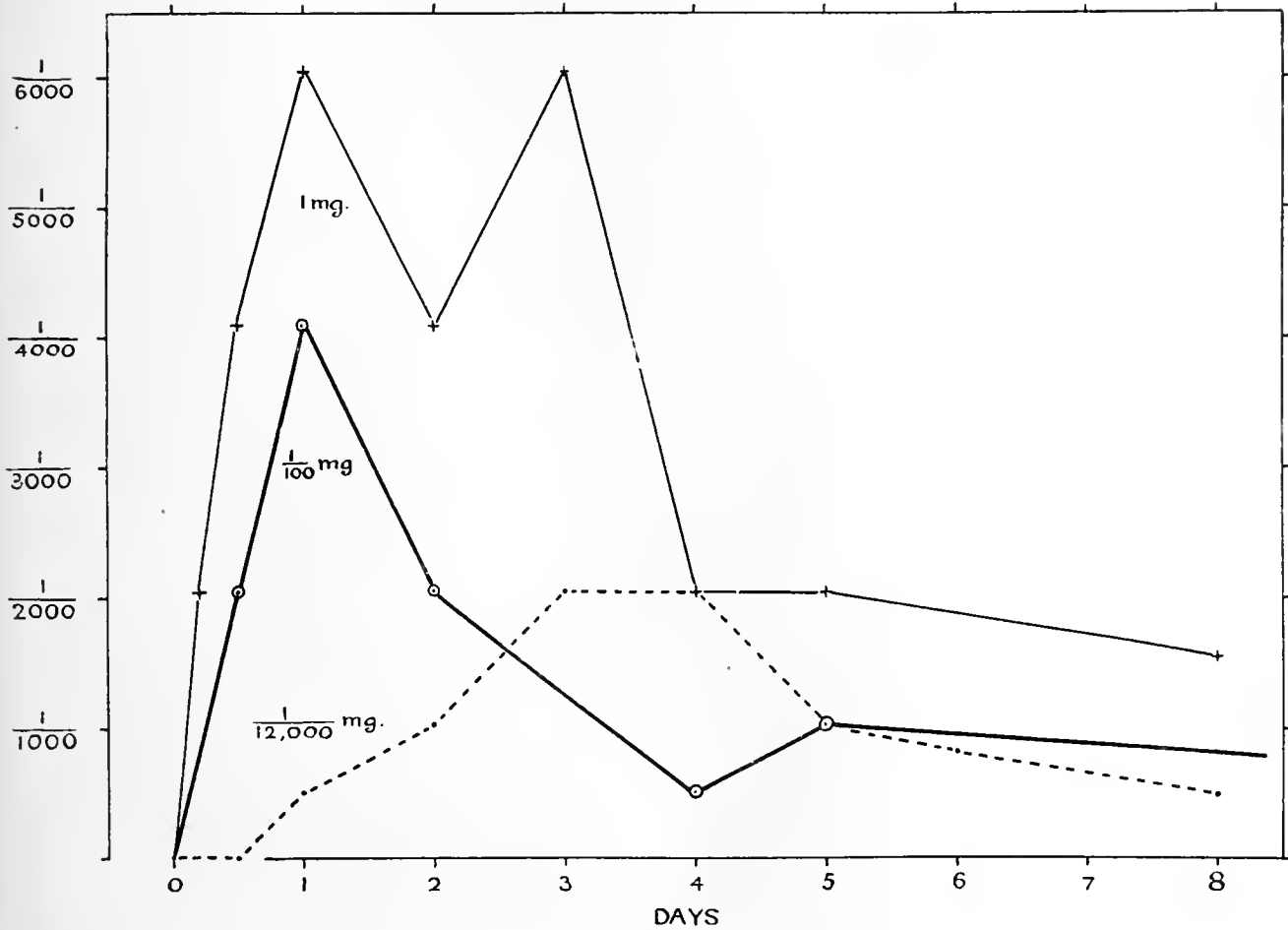


Fig. 2.

#### *Experiment IV.*

The ten rats in this series were given a very large dose of nucleoprotein, *i.e.* 1 mg. in 1 c.c. saline. In fresh solution this dose is usually fatal to rats, so the solution was incubated for 48 hours at 37° C. under toluol in order to allow a certain amount of hydrolysis to take place, which Rowland has shown reduces toxicity without interfering with the protective value of the nucleoprotein. In spite of this preliminary hydrolysis, four rats died during the experiment with symptoms of acute plague toxæmia.

The rats were bled 1 hr., 5 hrs., 10 hrs., 22 hrs., and subsequently every morning, after inoculation.

Before inoculation	1 hour	5 hours	10 hours	22 hours	2 days	3 days	4 days	5 days	8 days
—	—	1/2048	1/4096	1/6044	1/4096	1/6044	1/2048	1/2048	1/1536

The curve obtained in this experiment is higher than in the foregoing series, but this increase is not commensurate with the increased dose of vaccine given to the animals. A point has apparently been reached when increased dosage of vaccine does not bring about a proportional increase in opsonin production, as the amount of opsonin present is not much greater than with a tenth of the dose, as given in experiment I.

The drop in the curve after the sixth bleeding is remarkable and may be attributed possibly to the fact that two of the rats bled at this time died shortly afterwards of toxæmia. The curve rose after this, and the general tendency of the curve is towards a maintenance of the maximum for a longer period than in series I and II.

#### *Experiment V.*

Series II, which received a dose of  $\frac{1}{10}$  mg. of whole vaccine, was given a repeated dose of the same quantity ( $\frac{1}{10}$  mg.) thirty-five days later, when the index had been normal for some weeks. The following results were obtained.

Before inoculation	10 hours	1 day	2 days	3 days	4 days	7 days
—	1/4096	1/16384	1/24476	1/24476	1/16384	1/4096

It will be observed that in comparison with the curves obtained in series II, that the ordinates of the curve are very much higher than those obtained in the latter series, also that the optimum point is postponed to the right, taking place between the second and third days instead of between the first and second days.

Rats vaccinated with this dose retain their immunity for about a year. The opsonin production falls in the first week after inoculation, but the animals are still quite sensitive to a fresh dose of vaccine, and greatly increased response is elicited on re-vaccination.

#### *Experiment VI.*

In this experiment the washed bodies of the bacilli were employed for inoculation. The nucleoprotein was extracted in the usual way and the residue was washed and centrifuged down daily. This suspension

of bacterial bodies was kept under toluol to prevent decomposition. At the end of five days the supernatant fluid was quite free from precipitable nucleoprotein.

Each of a series of ten rats was inoculated with 0.9 mg. of these extracted bodies corresponding to a dose of 0.1 mg. whole vaccine and to 0.01 mg. of nucleoprotein. The serum of these animals was tested from day to day, but no increase in opsonic index was obtained throughout the experiment.

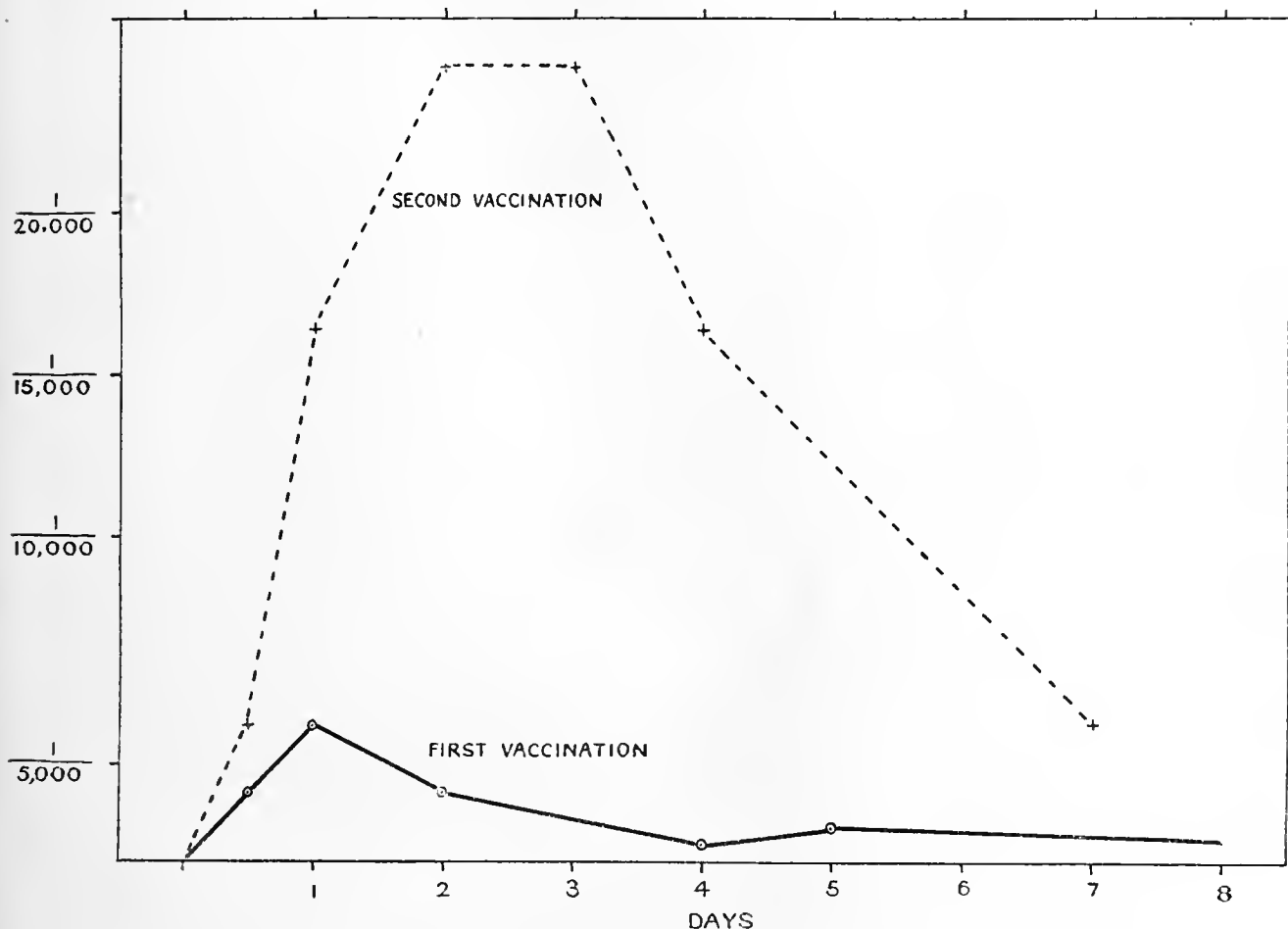


Fig. 3.

*Summary of rat experiments.*

From the study of the foregoing experiments the following facts appear to emerge. The production of opsonin in immune plague serum at first rises with the production of immunity, and soon returns again to normal leaving the resistance to infection (as ascertained by the direct test) still very high (see fig. 4). A second vaccination shows however that the capacity for response is much enhanced. In the

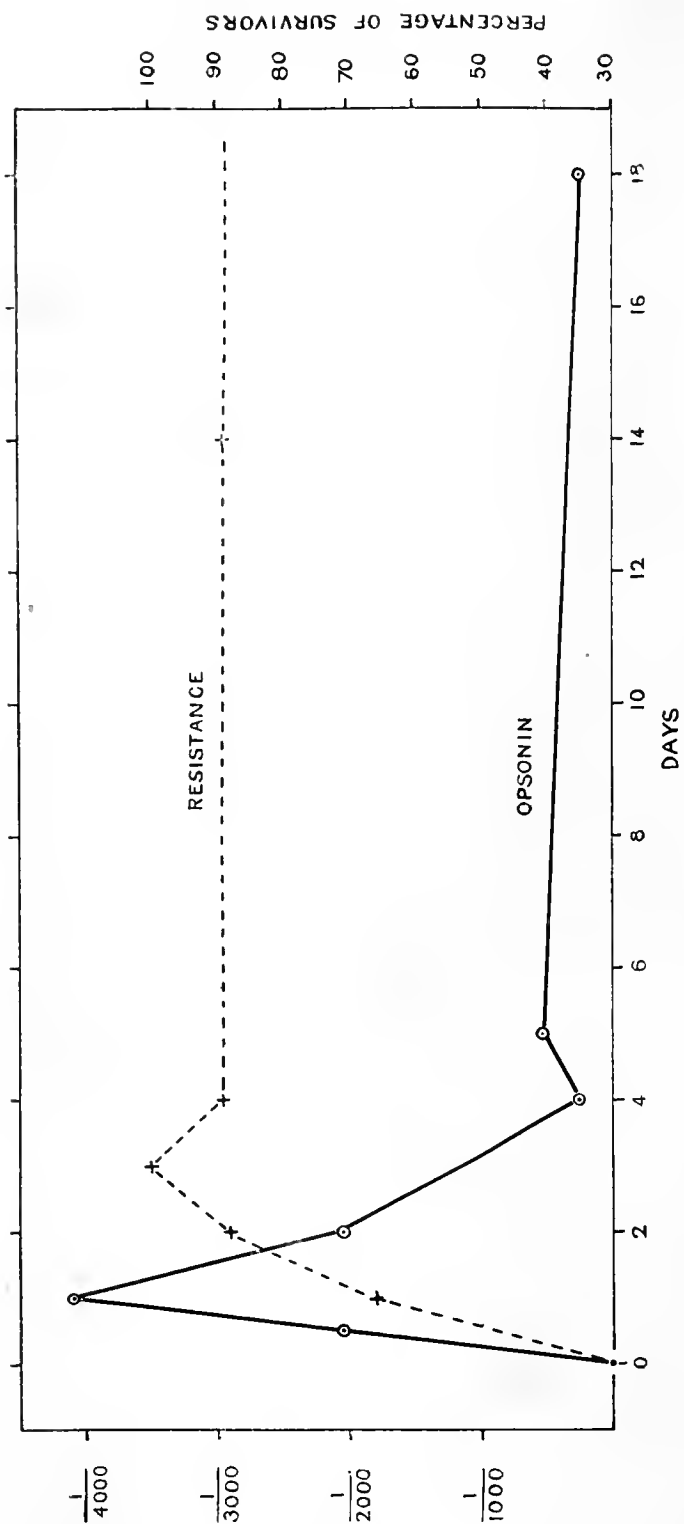


Fig. 4.

curves representing experiments I and II the maxima of the curves are reached during the first or second day by which time full immunity is established. In the case of the very small dose ( $\frac{1}{12000}$  mg.) in experiment III the maximum is postponed till the third day, and with the large dose (1 mg.) in experiment IV, the maximum is maintained for a larger period. The relative quantity of opsonin present is greater with the larger dose of vaccine used for inoculation.

In plague vaccination the active principle producing opsonic rise in the serum of rats resides in the nucleoprotein contained in the bacillus. This is shown (1) by the fact that the curves obtained by use of nucleoprotein solution and whole bacillus respectively are practically the same both as regards date of maxima and height of curve, and (2) by the fact that the washed bodies of the bacilli from which all the nucleoprotein has been extracted have no opsonin-producing value.

## B. HUMAN EXPERIMENTS.

### *Experiment I.*

I received  $\frac{1}{10}$  mg. nucleoprotein in 1 c.c. saline, which nucleoprotein had been hydrolysing under toluol for five days<sup>1</sup>. This solution was first tested on rats with the following results :

2 rats given .2 mg. each alive after 1 week.

„ „ .4 „ „ „

„ „ .6 „ „ „

„ „ 1.0 „ died in one and three days respectively with post-mortem evidence of plague toxæmia.

The arm at the seat of inoculation was somewhat stiff and painful for 48 hours. I suffered from a slight headache next morning but pulse and temperature were normal throughout.

The following results were obtained :

24 hours	2 days	3 days	4 days	5 days	6 days	7 days	9 days
—	—	1/1024	1/768	1/1024	1/1536	1/512	1/512

It will be observed that the maximum point of the curve in this case is very much later than in any of the curves obtained with rats, being on the sixth day instead of the first to third days.

### *Experiment II.*

The pooled serum of five persons who had been at some time previously inoculated with Haffkine's prophylactic and who had been

<sup>1</sup> This dose of nucleoprotein represents the amount of nucleoprotein contained in 1 c.c. of Haffkine's prophylactic.

given an inoculation of nucleoprotein solution by me was tested on eight successive days for opsonin after inoculation. The nucleoprotein solution was the same as used in the last experiment, but had been hydrolysing for 12 days.

Initials	Symptoms	Last dose of Haffkine's prophylactic
J. W.	Slight stiffness in arm and dizziness next morning, pulse and temperature normal.	About 12 months
C. C.	Slight stiffness in arm next morning, pulse and temperature normal.	„
S. R.	No symptoms, pulse and temperature normal.	Over 12 months.
I. R.	Slight stiffness in arm, pulse and temperature normal.	10 months.
F. W.	No symptoms, pulse and temperature normal.	About 8 months.

The following results were obtained with the pooled serum :

1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
—	—	1/512	1/512	1/1024	1/1024	1/512	1/512	1/256

It will be observed that the top of the curve is reached between the fifth and sixth days after which it gradually falls. No increase of opsonin was observed until the third day. The five persons inoculated were practically without symptoms throughout the experiment.

### Experiment III.

The pooled serum of five persons who had never been previously inoculated with plague vaccine and who had been given a dose of  $\frac{1}{10}$  mg. hydrolysed nucleoprotein, subcutaneously, were tested for opsonin. The nucleoprotein solution had been hydrolysing for eleven days. A dose of 1 mg. was non-lethal for rats.

Initials	Symptoms						
H. D.	Slight erythema at seat of inoculation, pulse and temperature normal.						
H. P.	„	„	„	„	„	„	„
A. S.	No symptoms.						
C. F.	„						
G. M.	„						
	Before inoculation	3 days	4 days	5 days	6 days	7 days	10 days
	Less than 1/32	1/128	1/128	1/256	1/384	1/256	1/192

In this series the opsonic response was not as great as in series II. The number of persons in each series is small and one would hesitate to draw any definite conclusion from these results, but as far as they go they tend to show that pre-existing sensibility has an influence on the opsonic index in human plague vaccination.



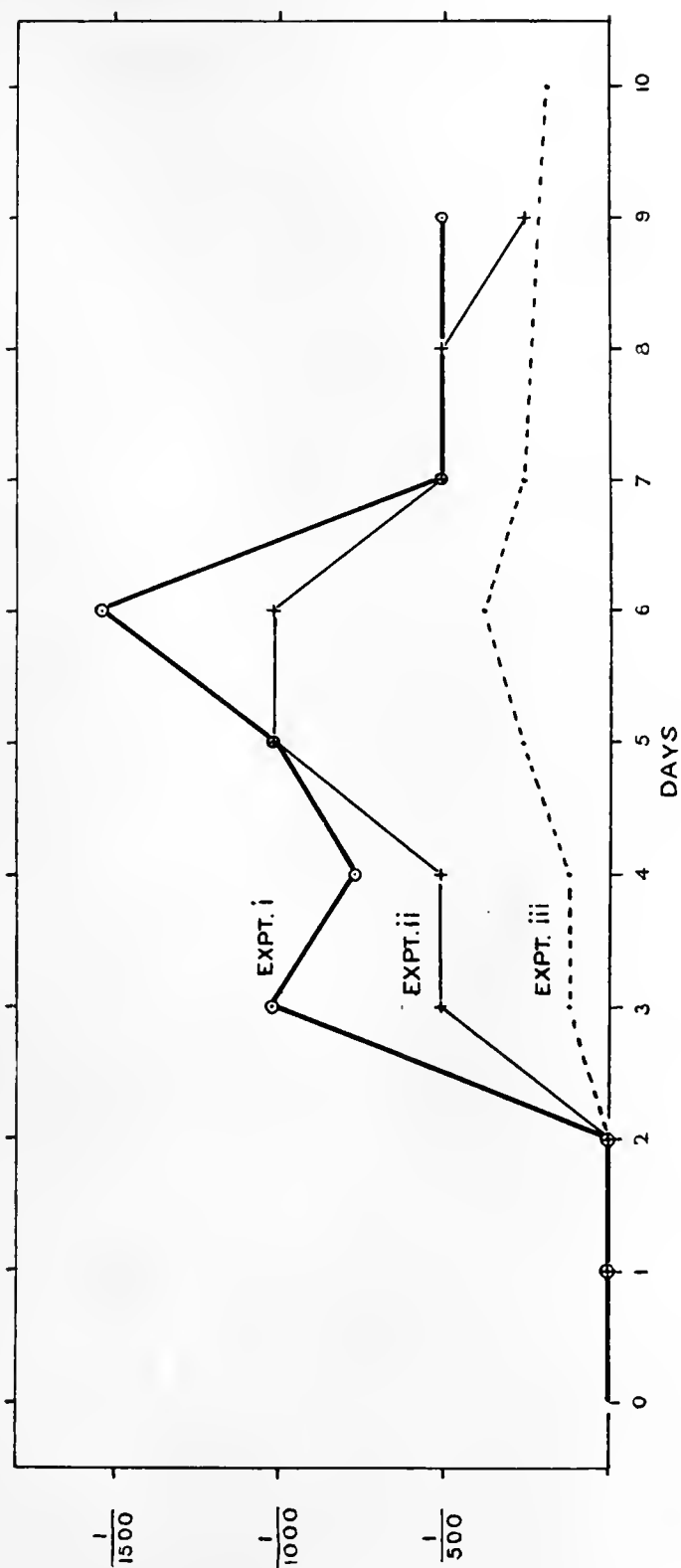


Fig. 5.

## CONCLUSIONS.

1. The substance which produces a rise in the opsonic index in immune plague serum is the nucleoprotein contained in the bodies of the bacilli.
2. The washed bodies of the bacilli when used as a vaccine do not cause any increase in the opsonic index.
3. Within limits, the larger the dose, the greater the opsonic response.
4. A second dose of vaccine raises the index above the maximum of the first inoculation, even after the curve has fallen to its normal level.
5. In the early stages, in rats, the opsonic response and the degree of protection aroused rise together.
6. In human beings the maximum response is observed to be much later than in the case of laboratory rats, and the response in a series of previously vaccinated persons is somewhat greater than in a series of persons not previously vaccinated.
7. The local and constitutional effects of nucleoprotein vaccination compare very favourably with those observed in other methods of protective inoculation.

I desire to express my thanks to Prof. C. J. Martin, F.R.S., Director of the Lister Institute, and to Dr J. C. G. Ledingham, Chief Bacteriologist, Lister Institute, for valuable advice and suggestions throughout the inquiry, and particularly to Dr Sydney Rowland, without whose constant advice and help the experiments could not have been carried out.

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### LXIII. THE PREPARATION OF ANTITOXIC PLAGUE SERA.

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THAT it is possible to obtain an antitoxic as distinguished from an anti-infectious plague serum was shown by Markl (1898, 1901, 1903) and other workers. The strongest serums have been those obtained by Dean (1906) and Rowland (1911). The former used for the immunisation of horses the filtrates from old (8—10 weeks) bouillon cultures and obtained a serum of which 1 c.cm. neutralised either 150 or 450 M.L.D. of the toxin according as one takes the death-time-limit of the M.L.D. to be 48 hours or 4 days. Rowland used a toxin extracted from the plague bacillus by means of his "sulphate process" and the serum so obtained was found to neutralise about 100 M.L.D. per c.cm.—the death-time-limit of the M.L.D. being 48 hours. In order to compare these two serums we must base our calculations on the 48 hour limit, and judging by this standard there is not much to choose between them. This 48 hour death-time-limit is the most convenient one to take as the *majority* of rats which have received an injection of plague toxin either die within 48 hours or live longer than 7 days. For this reason it was decided to take this limit in the following experiments and *for the purposes of comparison* to consider any animal alive on the 3rd day as cured of the intoxication due to the plague toxin. For therapeutic purposes a serum which kept animals alive for 3 days only would of course be useless, but experiments made with such a serum might well point out the road to the attainment of better results.

It seems advisable to call attention here to the fact that occasionally many rats which survived the acute attack and which had at the end of a week apparently quite recovered from the inoculation of the toxin-serum mixture, died later in a condition of marasmus. This may be due either to the failure on the part of the serum to neutralise some constituent of the toxin which comes into play after

the acute attack has passed off or, as some results suggest at first sight, to the serum itself possessing toxic properties. For example, the following mixtures of toxin and antitoxin were made (the volume in each case being made up to 4 c.c.), allowed to stand at room temperature for  $\frac{3}{4}$  hour and then injected into rats subcutaneously.

*Calcutta* (bleeding 3. 2. 12). Rats 90—95 grms.

2.1	mgm.	toxin	+	1	c.cm.	serum	=	†	within 48 hours
„	„	„	+	0.75	„	„	=	lived	
„	„	„	+	0.5	„	„	=	†	by morning of 5th day
„	„	„	+	0.25	„	„	=	lived	
„	„	„	+	0.1	„	„	=	lived	
„	„	„	+	0.04	„	„	=	lived	
„	„	„	+	0.02	„	„	=	†	within 48 hours
„	„	„	+	0.01	„	„	=	†	„ „

† = died

Now the M.L.D. of this toxin was found to be between 0.56 mgm. and 0.7 mgm. and 2.1 mgm. was considered to be equal to three available minimal lethal doses. We are then faced with the paradox that while 1 c.cm. of serum failed to neutralise 3 M.L.D. this amount of toxin was rendered ineffective by 0.1 c.cm. and 0.04 c.cm.

If this result be due to some toxic property inherent in the serum itself, then large doses of serum alone ought to have a similar effect. But this is not the case as I have injected subcutaneously into rats 10 c.cm. of an antiplague serum, which gave this “paradox-result,” without any ill effects. On another occasion 2 c.cm. of three other similar sera were injected subcutaneously into three rats. Two of these rats seemed ill on the second day, but they soon became quite well again. So it cannot be said that the serum itself is toxic in the doses given.

Besides what I have for convenience termed the “paradox-result,” there is another source of confusion in evaluating a serum. It has been shown by Rowland (1910, p. 544) that a certain dose of his virulent plague culture will when injected subcutaneously into rats kill on an average only 70 out of 100, *i.e.* with regard to this particular dose some 30% of rats are immune. As there is this natural immunity to infection, it is allowable to presume that the opposite condition may also occur, and that some rats may be more markedly susceptible than others to the influence of plague toxin. It is to these causes that are due the irregularities which not infrequently appear in the tests.

The result of the following experiment may be taken as showing the existence of an increased susceptibility,

0.553	mgm.	toxin	+	4	c.cm.	normal rat serum	=	lived
„	„	„	+	3	„	„	„	=died
„	„	„	+	2	„	„	„	=lived
„	„	„	+	1	„	„	„	= „

while the opposite condition, namely one of immunity, appears to be present in the case of the first rat in the series below :

1.66	mgm.	toxin	+	1	c.cm.	normal rat serum	=	lived
0.88	„	„	+	„	„	„	„	=died
0.76	„	„	+	„	„	„	„	= „

A good example of irregularities due to the causes above mentioned is furnished by the tests of a sample of serum from the horse *Lemberg*.

*Lemberg*. 10. 4. 12.

1st test 11. 4. 12. Rats weighing 95—110 gm. Inoculations subcutaneous.

2.1	mgm.	toxin (3—4 M.L.D.'s)	+	1/60	c.cm.	serum	=	lived
„	„			+1/80	„		=†	within 24 hours
„	„			+1/100	„		=	lived
„	„			+1/150	„		=†	within 36 hours

The animals that lived did not show any symptoms of illness and so it was concluded that a repetition of this test would show definitely what was the value of this serum.

2nd test 15. 4. 12. Rats weighing 90—100 gm. Inoculations subcutaneous.

2.1	mgm.	toxin	+	1/60	c.cm.	serum	=†	within 24 hours
„				+1/80	„		=†	„
„				+1/100	„		=†	„
„				+1/150	„		=†	„

It is obvious that this second test does not help us. We can only infer either that the rats in the second test were hypersusceptible or that two of the rats in the first test were immune. So it was necessary to test this serum a third time.

3rd test 18. 4. 12. Rats weighing 90—120 gm. Inoculations subcutaneous.

2.1	mgm.	toxin	+	1/20	c.cm.	serum	=	lived
„				+1/30	„		=	„
„				+1/40	„		=	„
„				+1/50	„		=	„
„				+1/60	„		=†	within 36 hours

We may now conclude that the antitoxic value of this serum is represented in the above test by  $\frac{1}{50}$  c.cm. and that consequently 1 c.cm. = 150 M.L.D.

An endeavour was made to obtain in the case of each sample tested a clean result such as that given above, but it was not possible to attain this end in every case. In those cases where a result was obtained

similar to that of the first of the above tests the serum would be valued at  $\frac{1}{60}$ th c.cm. or 180 M.L.D. per c.cm.; when the result was like that of the second test one could only say that the serum did not neutralise 180 M.L.D.'s per c.cm.

The next point to be considered is the toxin used as a test toxin. I have used various products as follows:

(1) *Filtrates* of broth culture are rarely toxic enough and are highly unstable. Thus in 29 trials, only twice was a filtrate obtained of which 0.1 c.cm. would kill a rat; in one instance a filtrate, which, when first tested, killed a mouse of 25 grammes within 24 hours in a dose of  $\frac{1}{100}$ th c.cm. subcutaneously, had no effect three days later on a mouse of the same weight in a dose of  $\frac{1}{15}$ th c.cm.

(2) *Solutions of the toxic nucleoproteid* give good results when used fresh, but they lose toxicity steadily (vol. XI. supplement, p. 35); ultimately they seem to become relatively stable, but the results then obtained with them are quite different from those obtained with the same serum against fresh toxin solution, and call to mind the suggestion that there may be more than one toxic substance in the plague bacillus.

(3) *A dry preparation of the toxic nucleoproteid* has also been tried but gave irregular results in the serum tests.

(4) The most constant preparation which I have tried has been obtained by taking a quantity of the dry mass formed by mixing the bacilli with anhydrous sodium sulphate, melting it by placing it in the incubator at 37° C., obtaining an even distribution of the bacilli by thorough stirring and shaking and then solidifying the mixture quickly by pouring it into a shallow basin cooled by ice. This "toxin rock" (TRk 1) may be broken up into small pieces and kept in a stoppered bottle in the dark. When required for use, a small quantity is weighed out, dissolved in 0.85% NaCl at 36° C., cooled to about 0° C. and filtered cold<sup>1</sup>.

In the experiments detailed below

"*fresh toxin*" = a freshly prepared solution of toxic nucleoproteid,

"*SR 2*" = an old autolysed solution of the same,

"*SRNP 1*" = the toxic nucleoproteid in the form of a dry powder,

"*TRk 1*" = the "rock" of sulphated bacilli.

In the following table are given the results of a series of tests of the same serum with various toxins.

<sup>1</sup> A second sample of this "rock" did not give such satisfactorily constant results.

# *Reports on Plague Investigations in India* 391

Serum Y 83. *Wealdstone* bleeding 28. 1. 11.

Date when tested	Toxin used as test toxin	No. of rat minimal lethal doses neutralized per c.cm. of serum
13. 4. 11	Bouillon filtrate 7 c.	500
28. 6. 11	SR 2	< 50
1. 7. 11	Fresh toxin	50 ? +
4. 7. 11	SR 2	30
13. 10. 11	"	< 2
16. 10. 11	Fresh toxin	160
28. 11. 11	SR NP 1	? 10 ? 0
1. 12. 11	"	? 20 ? 0
"	Fresh toxin	600
30. 1. 12	TRk 1	60 +
1. 2. 12	"	150

With the idea of bringing out the differences more plainly the details of one or two of these tests are given below.

Serum Y 83. Tested 1. 12. 11. Toxin SR NP 1. Rats weighing 90—105 grms.

*Serum.* 3 mgm. toxin + 1 c.cm. serum = † within 18 hours  
 " + 0.5 " = † " 48 "  
 " + 0.25 " = † " 48 "  
 " + 0.1 " = † " 6th day  
 " + 0.05 " = lived

*Controls.* 0.75 mgm. toxin = lived  
 0.5 " " = † within 48 hours

NOTE. The M. L. D. of this toxin usually lay between 0.75 mgm. and 0.5 mgm.

Serum Y 83. Tested 1. 12. 11. Toxin = fresh toxin. Rats weighing 85—120 grms.

*Serum.* 3 mgm. toxin + 1 c.cm. serum = † within 18 hours  
 " + 0.5 " = lived  
 " + 0.25 " = † 5th day  
 " + 0.1 " = lived  
 " + 0.05 " = † within 18 hours

*Controls.* 0.75 mgm. toxin = † within 18 hours  
 0.5 " " = † " "  
 0.25 " " = † " "  
 0.1 " " = † 48—60 hours  
 0.05 " " = † within 48 hours

Inoculations in each case were made subcutaneously. Toxin and serum in contact for  $\frac{3}{4}$  hour at room temperature before injection.

The tests of this serum performed on 30. 1. 12 and on 1. 2. 12 using as test toxin "toxin rock" TRk 1 were of quite a different order:—

30. 1. 12. Rats weighing 90 grms.

2.1 mgm. toxin + 1 c.cm. serum Y 83 = no symptoms, lived  
 " + 0.5 " " = " "  
 " + 0.25 " " = " "  
 " + 0.1 " " = " "  
 " + 0.05 " " = " "

1. 2. 12. Rats weighing 90—115 grms.

2.1 mgm. toxin	+ 0.05 c.cm. serum	= no symptoms, lived
„	+ 0.02 „	= ill, lived
„	+ 0.01 „	= † within 48 hours
„	+ 0.005 „	= † „ „

NOTE. The M.L.D. of toxin TRk 1 lay between 0.7 mgm. and 0.56 mgm. and nearer the latter than the former.

It is clear that toxin rock TRk 1 gave the cleanest results and as it acted similarly with other serums it was taken into use as a test toxin. We see also from the tests of Y 83 that neither the autolysed toxin SR 2 nor the toxic nucleoprotein powder SRNP 1 are suitable to be used as test toxins. That filtrates of bouillon cultures, which are in reality solutions of autolysed toxin, should give better results than a toxin such as SR 2 is peculiar, but is interesting when considered in connection with Kolle's statement (1903, p. 361) that neither Bern nor Paris serum nor the serum produced by the injection of centrifuged old bouillon cultures had any neutralising effect upon the toxin contained in old bouillon cultures, and that he could not confirm Markl's statement that it is possible to produce an antitoxin against the poison of old cultures. This behaviour of toxin SR 2 is very puzzling and recalls the suggestion that there may be two toxins produced by the plague bacillus, and that this may be the same kind of toxin as is spoken of by Kolle. Or Palandino-Blandini's (1905) statement may apply to toxin as well as cultures. He says that typhoid and plague serum protect against higher multiples of the M.L.D. when virulent strains are used than when weakened strains are used, because in the last case a larger comparative amount of bacterio-nuclein is injected and the bacterio-nuclein influences the protective power of the serum. This can only apply if there is a total destruction of toxin. But if we consider the test made on 4. 7. 11 another possible explanation is forthcoming. The details of that test are as follows:

Y 83 serum. Toxin SR 2. Rats weighing 90—95 grms.

3 c.c. toxin SR 2	+ 1.0 c.c. serum	= no symptoms, lived
„ „	+ 0.5 „	= „ „
„ „	+ 0.25 „	= ill but recovered
„ „	+ 0.1 „	= „ „
„ „	+ 0.05 „	= † within 24 hours

Serum and toxin in contact for  $\frac{3}{4}$  hour before injection.

This is a perfectly clean result and we can say definitely that 0.1 c.cm. of serum neutralized 3 c.cm. of toxin. Now at the time of the test the M.L.D. of this toxin was 0.75 c.cm. or a little less, but not



0.5 c.cm., and the test dose would contain 4 M.L.D. of which we can only count three as available for evaluating the serum, so that 1 c.cm. of serum would neutralise 30 M.L.D. But the original M.L.D. of this toxin was about 0.2 c.cm. which would make the test dose of 3 c.cm. equal to 15 M.L.D., and as this was neutralised by 0.1 c.cm. of serum the serum would be per c.cm. equivalent to 150 original M.L.D. This titre is the same as the serum had when tested against toxin TRk 1 which was an unmodified toxin.

This is rather against the existence of two separate toxins and also against an absolute destruction of toxin and suggests that, as in the case of diphtheria toxin, the plague toxin has become modified into "toxoid"—some  $\frac{3}{4}$  of each original M.L.D. having been thus converted.

Such a toxin, *i.e.* one that has become modified by being allowed to undergo autolysis, is capable of producing immunity and anti-toxin as is shown by the case of the horse *Bombardier* which was immunised with comparatively non-toxic toxin. When first prepared this toxin had an M.L.D. for rats of about 0.2 mgm., but after being kept for 4–6 weeks at 36° C. the M.L.D. had risen to about 3 mgm. The details of the immunisation are given below. The toxin was injected into the muscles of the neck just in front of the scapula.

*Immunisation of Bombardier.*

Date	Mgms. of toxin inoculated	No. of rat M.L.D.'s inoculated	Sample bleeding	Date of test	Toxin used as test	No. of rat M.L.D. neutralised per c.cm.
May 4, 1911	10	3—4	—	—	—	—
„ 16 „	20	7	—	—	—	—
„ 26 „	40	14	—	—	—	—
June 9 „	80	28	—	—	—	—
July 4 „	150	50	—	—	—	—
„ 13 „	300	100	—	—	—	—
„ 14 „	—	—	20 c.cm.	—	—	—
„ 20 „	—	—	„	20. 7. 11	SR 2	5
„ 27 „	600	200	—	—	—	—
„ 28 „	—	—	20 c.cm.	28. 7. 11	SR 2	5
„ 30 „	—	—	„	31. 7. 11	„	5
Aug. 1 „	—	—	„	1. 8. 11	„	5
„ 3 „	—	—	„	4. 8. 11	„	5
„ 4 „	520	170	—	—	—	—
„ 9 „	1000	333	—	—	—	—
„ 16 „	—	—	20 c.cm.	16. 8. 11	SR 2	30

From a study of this table we learn several things. Firstly we see that injections of this modified toxin can give rise to the production of antitoxin. The amount of antitoxin was at first quite as large and

finally much larger than that usually found in the serum of horses immunised according to Yersin's method, *i.e.* intravenous inoculations of living cultures.

Secondly, we see that the introduction of 600 mgm. in divided doses produced an antitoxin content which was not increased when 600 mgm. was given in one dose after an interval of 14 days, but that 1520 mgm. given in two doses with only a five-day interval increased the amount of antitoxin in the blood six times.

Lastly we see that the quantity of antitoxin in the blood was the same 24 hours after as it was before inoculation, and was found to be still at the same level when tested on the third, fifth and seventh day after injection. If this modified toxin caused a "negative phase" one would expect to find evidence of it 24 hours after the inoculation of such a dose as 600 mgm. (the growth from 171 Roux bottles). We may conclude that there is in this case either no negative phase or one with a duration of less than 24 hours. Now if such a large dose causes only a short negative phase or none at all, it is justifiable to presume that a small dose, such as would be used as a prophylactic measure, would not have any ill effect and consequently that such a modified toxin would be eminently suitable for use as a "vaccine."

It will have been noticed that during the immunisation of *Bombardier* samples of blood were taken at intervals of 24 hours, 3 days, 5 days and 7 days after the injection of 600 mgm. of modified toxin, and that the antitoxin content of the serum was the same on each occasion. The question then naturally arises "Does this condition obtain after doses of other toxins and at what time after inoculation does the serum contain the greatest quantity of antitoxin? When horses are immunised by means of intravenous injections of living cultures, it is usual to bleed about three weeks after the last injection, but the reasons for choosing such a late date in the case of living cultures need not necessarily hold good when toxins are used. So a series of samples of blood were taken at intervals of 7, 14 and 21 days after an injection and the serum tested for antitoxin. The results are given in the following table.

From a consideration of the table one would draw the conclusion that the antitoxin content of the blood does not vary much between the 6th and the 21st day. This condition proved most convenient as it enabled one to space the doses nicely and at the same time to take samples to ascertain the effect of each dose—thus a sample could be taken and then a dose given at weekly intervals.

Name of Horse	No. of rat M.L.D. neutralised per c.cm. of serum			Remarks
	7 days	14 days	21 days	
Lemberg	100 *	<120 †	<120 †	* Tested against bouillon filtrate 7 c. † „ „ fresh toxin
„	9	9	9	Tested against toxin SR 2
Whiteface	30	30	30	„ „ „
Calcutta	30	30	30	„ „ „
„	30	? 30	30	„ „ „
„	75	75	—	„ „ TRk 1
„	750	525 (12 days)	—	„ „ „
Wealdstone	30 (6 days)	? 30 (13 days)	60 (20 days)	„ „ SR 2
„	75	50	—	„ „ TRk 1
„	225	240 (12 days)	—	„ „ „

Proceeding in this manner with three horses which had already been immunised the results embodied in the following table were obtained:

Horse	No. of rat M.L.D. neutralised per c.cm. of serum					
	Before immunisation	7 days after 10 mgm.	7 days after 25 mgm.	7 days after 50 mgm.	7 days after 75 mgm.	14 days after 75 mgm.
Wealdstone	12	12	30	75	75	50
Calcutta	60	60	12	30	75	75
Lemberg	30	30	30	30	75	75

NOTE. The toxin used for immunisation was freshly prepared before each injection and the size of the dose is given in milligrammes of nucleoprotein as calculated from an analysis of a similar powder of "sulphated bacilli." The doses must be considered not as absolutely accurate but only approximate, for there are always slight differences in the nucleoprotein content of different batches of powder.

It appears that in the case of an immunised horse, which is resting after a bleeding, one inoculation of freshly prepared toxin has not much effect upon the antitoxin content of the blood—as obtains also in the case of diphtheria and possibly of other toxins. The effect of a 2nd, 3rd and 4th dose is somewhat variable when each dose is considered separately, but the summation effect is to stimulate the production of antitoxin.

The amount of antitoxin production in these three horses in consequence of the injection of 112 mgm. of fresh toxin was not great, and so they were given a rest and then inoculated with an autolysed toxin. From the experience gained with *Bombardier*, it was decided to try the effect of giving the doses at intervals of three days instead of a week but as the reaction following the second dose had not passed off by the third day, the third dose was given seven days after the second, and a sample taken to ascertain the effect of the first two doses.

Samples were also taken at various intervals after the third dose. The antitoxin content of these serums is given in tabular form below.

Horse	No. of rat M.L.D. neutralised per c.cm. of serum								
	8 days before inoculation	7 days after 100 and 200 mgm.	7 days after 300 mgm.	12 days after 300 mgm.	19 days after 300 mgm.	24 days after 300 mgm.	32 days after 300 mgm.	30 days after 300 mgm.	46 days after 300 mgm.
<i>Wealdstone</i>	50	150	225	240	300	—	300	90	90
<i>Calcutta</i>	75	300	750	525	360	450	300	300	120
<i>Lemberg</i>	75	210	300	300	450	—	240	150	150

NOTE. In the case of Lemberg owing to scarcity of toxin the third dose was only 100 mgm. instead of 300 mgm. as in the case of the other two horses.

We see that, just as in the case of *Bombardier*, doses of autolysed toxin at short intervals have produced a marked rise in the antitoxin content of the serum. The horses were bled 8 litres on the 12th day after the last injection, and in each case a slight improvement in the serum took place 7–12 days after bleeding. After that a gradual decrease occurred in the amount of antitoxin in the blood, until on the 46th day after the last injection the strength of the serum was only about double what it was before this series of injections was begun. On this date re-immunisation was commenced with a solution of toxin which had been kept at 36° C. for 1 month. This solution was made up to contain 1 mgm. of nucleoprotein per c.cm., and when fresh the M.L.D. for rats was about 0·7 mgm. After a month at 30° C. the M.L.D. had increased to 3 mgm. or 4 times as much as it was originally. The doses given were 30 mgm., 100 mgm. and 300 mgm., corresponding respectively to about 43, 148 and 430 original M.L.D. or 10, 33 and 100 M.L.D. of the autolysed toxin. The results were

Horse	No. of rat M.L.D. neutralised per c.cm. of serum			
	Before re-immunisation	3 days after 30 mgm.	7 days after 100 mgm.	9 days after 300 mgm.
<i>Wealdstone</i>	90	150	300	240
<i>Calcutta</i>	120	180	300	300
<i>Lemberg</i>	150	150	<180	240
<i>Kitasato</i>	60	60	60	90

Here again we see that the response to autolysed toxin is better than to fresh toxin. Of course it may be said that the doses of fresh toxin were not so large nor given in such a short time as those of the autolysed toxin, and that therefore the results are not comparable. Such criticism is quite fair and one would have liked to have avoided it, but from my experience I should say that such doses of fresh toxin

could not be injected at such short intervals because the reaction following inoculation would be too great to allow of its being done. But we may compare the toxins not according to volume in c.cms. or weight in mgms., but according to the number of M.L.D. given. If we value the toxins according to their M.L.D. at the time of inoculation there is not so very much difference between the doses. In the first series the total number of M.L.D. was about 160, in the second about 200 and in the third about 144 M.L.D. If however we value them according to the M.L.D. when freshly prepared the differences are great, and the respective numbers would be about 160, 850 and 600 M.L.D. The difference in the amounts of antigen given in the above three series of inoculations may possibly be made more striking if we calculate the quantity of growth which was required to produce the nucleoprotein. Rowland's analyses have shown that on an average the growth from 1 Roux bottle yields 3.5 mgm. of nucleoprotein, and taking this amount as the basis of our calculations we find that the number of Roux bottles used was

Horse	1st series	2nd series	3rd series
<i>Wealdstone</i>	47	198	123
<i>Calcutta</i>	47	198	123
<i>Lemberg</i>	47	132	123

and the results suggest that other things being equal the response to an inoculation is more dependent on the amount than on the absolute toxicity of the antigen introduced. This agrees with the opinion expressed by Kretz (1909, p. 26), that the effect of the toxin in the production of antitoxin (diphtheria) depends not so much on its direct toxic value as on its antitoxin neutralising power—old weakened toxin giving as good results as fresh toxin.

Besides the toxicity and amount of antigen given there is another factor which influences the result. As in the cases of diphtheria and of tetanus so in the case of plague, each horse does not respond alike to a given stimulus. This is well illustrated by the results of the immunisations of the two horses *Ramsay* and *Wealdstone*.

*Ramsay* had between the 20th July 1910, and the 19th October 1910, 1340 mgm. of *fresh* toxin equal to about 4000 rat M.L.D. or the growth from some 380 Roux bottles, and yielded a serum of which 1 c.cm. neutralised some 400 rat M.L.D. *Wealdstone* had the first injection on Aug. 24, 1910, and by Oct. 10 had received 200 mgm. of fresh toxin. On Oct. 19th the serum had a titre of 150 M.L.D. per c.cm. The immunisation was continued, and by Jan. 7, 1911, the total quantity of toxin introduced was 3000 mgm. or 9000 rat M.L.D. or the

growth from some 860 Roux bottles. On Jan. 28, 1911, the titre of the serum was per c.cm. 500 M.L.D. when tested against a toxic bouillon filtrate, but only some 150 M.L.D. when tested against "fresh toxin." For our present purpose we will take the larger of these two values and comparing it with that of *Ramsay* we see that doubling the amount of antigen has only raised the titre of the serum by a fraction and that therefore the "individuality" of the horse has a great deal to do with the response which follows the introduction of the plague antigen. *Wealdstone* had still larger doses of toxin—1000 mgm., 2000 mgm., 2000 mgm. and 3000 mgm. in single doses without any resulting increase in the titre of the serum.

We came therefore to the conclusion that it was useless to increase the size of the doses of fresh toxin to any great extent, not only because they seemed to be ineffectual in increasing the antitoxin content, but also because the local and constitutional reactions were so great that the intervals between the inoculations had to be lengthened from the 4 or 5 days usual at the commencement to one month at the end.

Both fresh and old toxin, but particularly the former, when given in doses of 100 mgm. and upwards cause a loss of flesh which is not to be accounted for by the temporary loss of appetite and the febrile condition brought about by the injection.

To sum up we may say that in the case of horses which have been already immunised, old weakened toxin appears to stimulate the production of antitoxin better than fresh toxin, and it has the great advantage of not interfering so much with the well-being of the animal, cf. *Pane*, 1911, p. 279.

In these tests the toxin and serum have been mixed and allowed to remain in contact for about  $\frac{3}{4}$  hour at room temperature before injection into the rats. Other workers however have used a higher temperature and allowed a shorter or a longer time for union to take place, and so experiments were made to ascertain the effect of these two factors. Toxin and serum were mixed, the volume when necessary made up to about 4 c.cm., and the mixture allowed to stand at 36° C. for  $\frac{3}{4}$  hour and for 1½ hours. Control doses of toxin alone were also diluted and kept at 36° C. for the same time. There was no difference in the results following the injection of mixtures or pure toxin kept at room temperature or at 36° C. for  $\frac{3}{4}$  hour or for 1½ hours.

The next point considered was whether other antisera possessed the power of neutralising plague toxin to any great extent. For this purpose the following tests were performed.

*Various antisera.*

1 c.cm. of serum was mixed with decreasing amounts of plague toxin, made up to about 4 c.cm. when necessary, allowed to remain at room temperature for  $\frac{3}{4}$  hour and injected subcutaneously into rats. The M.L.D. of the toxin used was between 0.7 mgm. and 0.56 mgm. The numbers show the weights of the rats in grammes.

Name of horse	Variety of serum	Date of bleeding	Date of experiment	Amounts of toxin			
				2.1 mgm.	1.4 mgm.	0.7 mgm.	0.56 mgm.
<i>Tasmania</i>	Pooled serum of 5 normal horses	28. 6. 11	12. 2. 12	+ 90	+ 120	+ 120	lived 120
<i>Melbourne</i>		29. 6. 11					
<i>Adelaide</i>		29. 6. 11					
<i>Hobart</i>		29. 6. 11					
<i>Wellington</i>		29. 6. 11					
<i>Bangor</i>	Antitetanic serum	9. 1. 12	12. 2. 12	+ 90	+ 90	+ 90	+ 90
<i>Vignette</i>	Had had injections of living streptococci and also of diphtheria toxin	19. 1. 12	12. 2. 12	+ 90	+ 95	lived 90	lived 90
<i>Maude</i>	Antistreptococcic serum	1. 11. 11	12. 2. 12	+ 90	+ 90	lived 90	lived 95
<i>Arkwright</i>	Anti pseudotubercle serum	10. 6. 8	19. 2. 12	+ 100	+ 90	+ 90	lived 90
<i>Broncho</i>	Antidysentery (intravenous inoculations)	26. 7. 6	28. 2. 12	+ 105	+ 110	+ 100	+ 100
<i>Nobby</i>	Antidysentery (intramuscular inoculations)	28. 2. 12	4. 4. 12	+ 105	lived 120	lived 120	lived 115
„	Antidysentery (intramuscular inoculations)	29. 3. 12	4. 4. 12	+ 120	lived 125	lived 120	lived 120
<i>Rich</i>	Antistaphylococcic	21. 2. 07	28. 2. 12	+ 90	+ 100	+ 100	lived 105
<i>Whitestreak</i>	Antidiphtheritic (1300 units)	8. 2. 12	28. 2. 12	+ 115	+ 115	+ 100	lived 105
<i>White Lady</i>	„ (150 units)	12. 1. 11	28. 2. 12	+ 120	+ 120	lived 120	+ 120

*Antiplague serum.*

2.1 mgm. of plague toxin were mixed with decreasing amounts of antiplague serum, made up to 4 c.cm. where necessary, allowed to remain in contact  $\frac{3}{4}$  hour at room temperature and then injected into rats subcutaneously.

Name of horse	Date of bleeding	Date of test	Amounts of serum					
			1 c.cm.	0.5	0.25	0.1	0.02	0.01
* <i>Corbally</i>	22. 1. 09	28. 2. 12	lived 90	lived 100	lived 110	+ 110	—	—
* <i>Whiteface</i>	15. 2. 09	23. 2. 12	„	lived 95	+ 90	+ 90	—	—
† „	26. 2. 12	26. 2. 12	„	lived 90	lived 90	lived 90	—	—
<i>Ramsay</i>	5. 11. 10	28. 2. 12	—	—	—	—	lived 115	lived 90
<i>Wealdstone</i>	14. 3. 12	20. 3. 12	—	—	—	—	lived 90	+ 100

\* Immunised with living cultures given intravenously.

† „ „ filtrates intramuscularly.

From these results we are justified in concluding that neither normal serum nor any of the ordinary antisera are capable of neutralising plague toxin to any appreciable extent compared with anti-plague serum.

Having determined that the antitoxin in antiplague serum is a specific antitoxin two questions naturally occur to one. Does it retain its antitoxic power for any length of time unimpaired? What effect will tropical temperatures have upon it?

As regards these questions Chourouppoff (1909) examined the protective and curative properties of ordinary antiplague Yersin serum of various ages up to 10 and 11 years. He found the 11 year old serum useless and thought the loss of power might be due to the 0.5% phenol present. From his experiments he concluded that if serum be heated at 55° C. on each of three successive days for 1½ hours and then be kept in the cold, in the dark and in the absence of air it will preserve its curative and protective properties unchanged for six years. Wladimiroff (1898) states that antiplague serum keeps up its strength for months and is not affected by passing through the tropics. Our tests do not go back far enough to enable us to speak of years, but we have the results of tests performed by Rowland and myself with an interval of some 18 months to 2 years between them.

The results are given in the following table:

Name of horse		Method of immunisation	Date of bleeding	Tested in 1910 by S.R. 1 c.cm. serum =	Tested in 1912 by A.T.M.C. 1 c.cm. serum =
<i>Corbally</i>	Y 65	Living cultures intravenously	22. 1. 9	10 M. L. D.	12 M. L. D.
<i>Whiteface</i>	Y 68	" " "	15. 2. 9	5—10 "	6 not 12 M. L. D.
<i>Kitasato</i>	Y 72	" " "	16. 12. 9	" "	12 not 30 "
<i>Whiteface</i>	Y 74	L. I. V. + toxin	20. 12. 9	40+ "	60 not 90 "
<i>Lightfoot</i>	Y 75	Toxin only	29. 12. 9	84 "	90 M. L. D.
"	Y 76	" "	4. 1. 10	125 "	30 "
"	Y 77	" "	18. 10. 10	66 "	30 not 60 M. L. D.
<i>Ramsay</i>	Y 79	" "	5. 11. 10	450 "	300 not 450 "

In these tests the M.L.D. of the toxin was about 0.7 mgm. or a little less, and the test dose was 2.1 mgm. The quantities of serum used were in the following descending scale: 1 c.cm., 0.5, 0.25, 0.1, 0.05, 0.02, 0.01 and 0.0066. This will explain why no values are given between 30 and 60 or between 300 and 450. These results show that plague antitoxin retains its value fairly well for 18 months to 2 years.

To ascertain the effect of heating on the antitoxin some small serum vials were filled with antiplague serum, sealed off in the flame and then heated in a water bath for ½ hour at 56° C. on each of three successive days.



Name of horse	Date of bleeding	Antitoxin content in M.L.D.	
		Before heating	After heating
<i>Wealdstone</i>	3. 2. 12	75 M. L. D. per c.cm.	75 M. L. D. per c.cm.
<i>Calcutta</i>	3. 2. 12	30 „ „	75* „ „
<i>Lemberg</i>	3. 2. 12	30 „ „	30 „ „

\* This appears to be an instance of a rat being hyperresistant. In the test before heating the rats which received  $\frac{1}{25}$  c.cm.,  $\frac{1}{50}$  c.cm. and  $\frac{1}{100}$  c.cm. of serum all died and in consequence it was not thought to be worth while to test the serum after heating beyond  $\frac{1}{25}$  c.cm.

It is obvious that heating antiplague serum under the conditions described has no deteriorating effect upon the neutralising power for plague toxin.

#### *Concentration of antitoxin.*

As the titre of the serums obtained has not been very high an attempt was made to increase the value of a serum by the Gibson concentration process which has proved so successful in the case of diphtheria antitoxin. Five hundred cubic centimetres of serum were precipitated with an equal volume of half-saturated solution of ammonium sulphate. The precipitate was collected and extracted with brine. Eleven hundred (1100) c.cm. of brine filtrate were obtained and precipitated with 0.25% of acetic acid. The precipitate was collected, made alkaline with powdered washing soda and dialysed against tap water. When found comparatively free from salts the contents of the dialyser—200 c.cm.—were filtered first through chain cloth and then through a small Berkefeld filter candle.

The antitoxic values at the three stages were:

Original serum	500 c.cm.	90 M. L. D. per c.cm.
Brine extract	1100 „	45 „ „
* Concentrated serum	200 „	150 „ „

\* Unfortunately the serum became contaminated in the dialyser and perhaps some of the loss may have been due to this cause.

There is thus an increase in the antitoxic value per c.cm. to the extent of  $1\frac{2}{3}$ , but on the whole a loss of 33% of antitoxin.

#### SUMMARY.

1. It is possible to obtain a plague antitoxic serum by means of injections of toxin obtained from the plague bacillus by Rowland's sulphate process. So far however the antitoxic value has not been high. The usual neutralising power has been 300—400 rat M.L.D. per c.cm. and on only one occasion was a value of 750 M.L.D. reached.

2. In an immunised horse one obtains a better response, as regards antitoxin production, if one uses an old weakened toxin instead of a fresh toxin.

3. Plague serum retains its antitoxic properties for months unimpaired.

4. Heating at 56° C. for  $\frac{1}{2}$  hour on each of three successive days has no effect on the antitoxin.

5. Plague antitoxin may be concentrated by the same process that is used for concentrating diphtheria antitoxin.

I desire to take this opportunity of thanking my colleague Dr S. R. Rowland for so generously placing at my disposal the large amounts of toxin which have been required. To my chief, Dr C. J. Martin, I am greatly indebted for much kindly and helpful criticism.

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3  
LXIV. THE INFLUENCE OF CULTIVATION IN SERUM-CONTAINING MEDIA UPON THE VIRULENCE AND IMMUNISING PROPERTIES OF THE PLAGUE BACILLUS.

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(With Plate XVI.)

I. INTRODUCTION.

IN the first report on investigations into plague vaccines (Rowland, 1910) it was shown that an effective immunity against a virulent broth culture of *Bacillus pestis* can be aroused in white rats by a single injection of an extract obtained from bacilli propagated in broth or broth agar. The extract, which consists for the most part of nucleoproteins, was obtained by desiccating a bacterial paste with anhydrous sulphate of soda, and subsequently extracting the soluble portion with a dilute solution of the salt. The residue left after the extraction was devoid of immunising properties.

Further observations led to the discovery that, notwithstanding this satisfactory protection of rats against virulent broth-cultivated microbes, the protective power was only of a low order when tested against an organism grown in blood serum.

The virulence of *Bacillus pestis* is in certain cases considerably increased by propagation in serum previously heated to 55° C., but this is not the whole nor, I believe, the principal explanation of the results, for, as will be shown below, good immunity against both broth and serum bacilli follows the injection of the same quantity of nucleoprotein prepared from organisms grown in serum. It appears, therefore, that growing in serum as well as enhancing virulence, modifies the antigenic properties of the bacillus.

There are several observations recorded showing that the antigenic properties of an organism, as tested by serological methods, may be altered according to the medium in which it is grown. Grassberger and Schattenfroh (1905) found that the serum of an animal prepared by injection with the heated serous exudate from a case of quarter-ill agglutinated the *Bacillus chauvei* when grown in this medium, but not

when habituated to ordinary laboratory media. Altmann and Rauth (1910) were able to modify the antigenic properties of *Bacillus coli* by propagating it upon media containing phenol or arsenic, whilst the cultural and fermentation characters remained unaltered. In both arsenic and phenol media a complete alteration in the receptors of the bacillus, as determined by the complement deviation method, was brought about. The serum of an animal injected with either an arsenic or phenol strain possessed "ambo-ceptors" only for the appropriate modified strain, but none for the original, and *vice versa*. Once the variation was established it remained permanent even when the altered strain was subsequently grown on ordinary agar.

Bordet and Sleeswyk (1910) observed similar facts with the organism of whooping-cough, discovered by Bordet and Gengou. This organism grows well on blood agar, but with difficulty on ordinary agar. By educating the organism to grow on a broth-agar medium containing less and less blood, a strain was ultimately obtained which grew well with no blood present. The serum of a horse immunised against "blood-microbes" agglutinated in high dilution the bacillus so propagated, but not the same strain after it was habituated to grow on broth-agar. A similar serum, prepared by inoculating a broth-agar strain, whilst agglutinating in high dilution the homologous strain, failed to agglutinate the "blood-microbes." The agglutinin of serum prepared by injection of "blood-microbes" was absorbed by both strains, but the agglutinin of a serum prepared by broth-microbes was only absorbed by the bacilli of the corresponding variety.

Bordet and Sleeswyk also made experiments with the complement-deviation method of Bordet and Gengou, but the sera of immunised animals did not afford a means of distinguishing between the original and the modified strain. This could, however, be done with the sera of patients convalescent from whooping-cough, such sera agglutinating only the blood-microbes. By using the method of conglutination (Bordet and Gay, 1906), however, they obtained results indicating that the receptors of the bacilli had undergone a modification under the influence of the changed nutritive environment, even in those cases where the classical method of Bordet and Gengou failed to afford evidence of differentiation.

The bearing of these observations in practice will be obvious. In the case of a flea-borne epidemic of plague we are probably concerned with strains of bacilli grown in serum. The source from which the flea draws its infection is the septicaemic blood of the rat, and during the

time that the flea preserves plague bacilli in its stomach and intestine, these are growing in serum more or less altered by the digestive juices of the insect. The habits of the flea ensure a constant supply of fresh serum to replace that digested or voided.

II. *The relative virulence of B. pestis grown in various media, viz. broth, fresh serum and serum heated to 55° C.*

The plague bacillus which has previously been propagated on ordinary laboratory media will only grow with difficulty in fresh horse-serum. If bacilli from an agar culture be inoculated into fresh horse-serum kept at 37° C. and samples be observed at intervals under dark ground illumination a proportion of the bacilli will be found to undergo lysis in the serum. The photomicrographs reproduced in Plate XVI represent the appearance of the bacilli as seen in the counting chamber employed in these investigations. The method of dark ground illumination is that described in a previous report (this *Journal*, Vol. XII. p. 362). Both healthy bacilli growing in chains or in long forms (Figs. 1 and 2) as well as bacilli undergoing lysis (Figs. 3 and 4) are represented.

Lysis is dependent on the presence of some substance in the serum which is destroyed by heating to 55° C. for half-an-hour, for in serum thus treated the preliminary lysis is nearly abolished and multiplication begins much earlier. After the lysis is over the survivors commence to multiply. If they be now transferred again to fresh horse-serum the preliminary lysis is much less and multiplication commences earlier.

The virulence of the strain after propagation in broth, fresh normal horse-serum and serum previously heated to 55° C. for half-an-hour, was tested by comparing the proportion of rats which succumbed to the subcutaneous injection of 5,000,000 bacteria grown in the various media. The broth culture employed killed 49 out of 67 (73%) of the rats when this number of bacteria was injected.

It was anticipated that the capacity to flourish in fresh serum of the rat might be accompanied by an increase in virulence, but to my surprise it was found that the contrary had occurred, and, out of 10 rats inoculated with 5,000,000 bacilli, only two succumbed. The experiment was repeated several times with the same result.

To test the virulence of the organisms grown in horse-serum, heated to 55° C., two series of 20 rats each were inoculated with 5,000,000 bacilli. The whole 40 rats succumbed to plague.

In another series of experiments the broth organisms were cultivated

first in horse-serum which had been heated to 55° C. for half-an-hour. The change from broth media to heated serum was, as before, accompanied by a considerable rise in virulence. After cultivating for one day in heated serum, a seeding was transferred to fresh rat-serum and incubated at 37° C. The organisms commenced to multiply in a few hours without any considerable preliminary lysis, but, when tested for virulence, proved to have become attenuated to a striking degree—5,000,000 bacilli now killing only four rats out of ten. By the intermediate culture in heated horse-serum, a race of high virulence and also largely insusceptible to the lytic action of fresh normal serum had developed, but on subsequent culture in the fresh serum the virulence, instead of being maintained or enhanced, was, as with bacilli directly transferred from broth, markedly attenuated.

The attenuating effect associated with the growth of broth-organisms in fresh serum was always observed, but in the case of bacilli previously propagated for a few days in heated serum, although attenuation was the rule, it was not absolutely constant<sup>1</sup>.

In the meantime I might mention that fresh serum does not produce this effect to anything like the same extent upon organisms derived directly from the body of an animal infected with plague.

It seems, therefore, that when broth-organisms are sown in fresh serum in sufficient amount to ensure that some of the individuals escape lysis, the survivors and their progeny are reduced in virulence, whereas the same broth-organisms cultivated in serum heated to 55° C. are little lysed and develop a highly virulent race. Observations presenting a partial analogy to the above were made by Ainley Walker (1903), who found that cultivation of *B. typhosus* in immune serum led to rise of virulence; Ainley Walker attributed this to the selection of a strain of bacilli immune to the ambo-ceptor.

*The constituent of the heated serum responsible for the rise  
in virulence.*

If the serum be boiled, and from the protein clot there be squeezed out the contained liquor, this liquor will not sustain growth of plague bacilli to any extent. Some protein constituent of the serum is apparently necessary to maintain growth. This conclusion is supported by the fact that if serum albumen be prepared in its crystalline

<sup>1</sup> On account of the possible importance of this observation and its bearing on the general question of immunity it is being investigated and will form the subject of a further report.

condition from horse-serum, the crystals dialysed free from salts and the resulting protein solution added to Fraenkel's synthetic medium, the protein being present to the extent of  $\frac{1}{2}\%$ , a solution is obtained in which the plague bacillus grows luxuriantly. At the same time the virulence is well maintained. 5,000,000 organisms grown in this medium were inoculated into each of ten rats, nine of which died of plague. The conclusion that the serum albumen is essential was confirmed by the fact that if, from a solution prepared as above, the albumen be filtered off by means of a Martin gelatin filter, plague bacilli will not grow in the filtrate.

III. *Antigenic properties of B. pestis grown in various media; degree of immunity obtained against a serum strain.*

A. *By vaccination with an antigen prepared from bacilli grown in broth.*

Forty-seven rats were immunised by an antigen prepared from broth-grown bacilli by the method described in my first Report (1910); they received subcutaneously 0.05 mg. of the nucleo-protein dissolved in salt solution. Five days later when, from the results of previous work (1912, p. 369) immunity may be assumed to be fully established, 22 of these vaccinated rats received the test dose of living pest grown in the standard way in broth. Of these rats 17 or 77% survived the inoculation—a result in accordance with previous experience of the vaccinating power of the nucleo-protein extract. At the same time the remaining 25 rats received a similar dose of the same strain grown in heated serum. Only 7 or 28% survived. This result, it must be confessed, was at the time disquieting. The conclusion to be drawn from this experiment is that immunisation with an antigen prepared from a strain grown in broth affords but little immunity against the same strain grown in serum.

B. *By vaccination with living cultures.*

The first point to determine was whether it was possible to immunise against infection by the race of enhanced virulence, such as is produced by growing in heated serum. Kolle and Otto (1903 and 1904), and Strong (1906 and 1907), showed that the highest grade of immunity that it is possible to confer on experimental animals follows the inoculation of living pest. The preparation of a series of rats immunised by living virulent pest was, therefore, proceeded with. For this purpose a series of rats received a preliminary inoculation of the nucleo-protein

extract with the object of enabling them to withstand the subsequent inoculation of the living culture. Seventeen rats survived the second inoculation. These rats having first received nucleo-protein from the broth-agar grown bacillus, and then living virulent pest, may be considered immunised to as high a degree as is possible. Twenty-four days after the injection of the living organisms each rat received 5,000,000 bacilli from a culture grown in heated serum, a dose which killed the whole of 20 unprotected rats and 72% of rats previously vaccinated with nucleo-protein extract prepared from broth culture. Of the series inoculated with living culture every one survived the inoculation. It is therefore possible to immunise against the enhanced virulence of the serum race by the employment of living cultures.

C. *By vaccination with an antigen obtained from organisms grown in media containing serum.*

In the first instance a trial was made of a whole vaccine killed by heat, after the manner of Haffkine, with the difference that the bacilli were grown in serum instead of in broth. Two vaccines were prepared, one from a growth of the bacillus in unheated horse-serum, the other from the same organism grown in serum that had been heated to 55° C. The organisms were killed by a preliminary heating to 60° C. for half-an-hour. Twenty rats received such a quantity of each of these vaccines as contained 200 million organisms<sup>1</sup>. Six days afterwards each rat (40 in all) received the standard dose (5,000,000) of virulent organisms grown in heated serum. Not one survived. The failure of the heated serum race to vaccinate against the living serum race may have been due to the dose being too small, or to the deleterious effects of heat on the antigen contained in the bacilli. Both these objections to the heated whole vaccine could be met by employing the nucleo-protein extract prepared from bacilli grown in serum, and to this end efforts were made to procure a sufficient growth of bacilli in serum, from which to obtain the nucleo-protein.

This was accomplished by using Roux flasks, as in the technique already described (Rowland, 1910) for the preparation of the nucleo-protein from bacilli grown on broth-agar. The Roux flasks were prepared as usual with broth-agar, and to each was added a few c.c. of normal horse-serum sterilised by filtration and heated to 55° C. With this addition the bacilli grew well, and from the growth thus obtained

<sup>1</sup> About 10,000,000,000 organisms yield 1 mg. nucleo-protein.



the nucleo-protein was prepared in the manner already described by the use of anhydrous sulphate of soda.

The toxicity of this extract for rats was about the same as that of the extract previously prepared from organisms cultivated in broth-agar (Rowland, 1910, p. 555). Inoculated subcutaneously 0·8 mg. killed six rats out of six; 0·4 mg. also killed six rats out of six, and 0·2 mg. killed three rats out of six. 0·1 mg. failed to kill.

The immunising value of this extract when tested against serum-grown organisms was determined as follows: 24 rats received 0·02 mg. subcutaneously. Seven days later each rat received the test dose of living organism grown in heated serum. At the same time ten unprotected rats were inoculated with the test dose, all of which died. Of the 24 vaccinated rats 16 survived the test dose. In a second experiment made a month later 20 rats were inoculated with 0·02 mg. of extract made from bacilli grown on serum media and tested in the same way as in the former experiment. Seventeen survived the test inoculation, whilst of 20 controls all died. If we combine these two experiments they show a survival rate of 75%, which is comparable with that previously obtained, where the broth race was employed both to vaccinate and to test the immunity. It is thus possible to immunise against a serum race of organisms, notwithstanding its enhanced virulence, provided the vaccine be prepared from organisms which have themselves been propagated upon sera. The results appear inferior to those obtained by vaccination with a living culture, but are not quite comparable, as in the latter case the less resistant rats were eliminated during the process of the immunisation.

In another experiment the organisms used for the test dose were obtained direct from a rat dead of plague and propagated in a mixture of broth and heated serum in which medium their virulence is well maintained. The antigen employed was obtained from organisms grown on agar to which heated serum had been added as in the previous experiments. The experiment was also modified to ascertain the influence of a second dose of the antigen upon the resistance developed by the rats. The nucleo-protein was given in doses of 0·05 mg., and the interval between the doses was one month. Fourteen days after the second injection the rats, together with 20 controls, were inoculated with 5,000,000 bacilli. The controls all succumbed. Of 74 rats which received but one dose of the nucleo-protein 35 survived the test dose (47%). Of 14 rats which received two protecting doses of the nucleo-protein, 11 survived the test dose (86%). The effect of

repeating the vaccination would appear from this experiment to double the immunity. The degree of protection afforded by the single injection is, however, much less than in the previous experiments. Further experiments made on this point show that a culture in serum-broth is more uniformly virulent than a culture in serum. This fact may serve to explain the smaller number of survivors (47%) amongst the rats tested with the serum-broth strain as against the number of survivors (75%) tested with the pure serum-grown organisms<sup>1</sup>.

To determine whether the antigen prepared in the above manner is equally or more effective against a race of organisms propagated in broth, the following experiment was made: 26 rats were immunised with 0.1 mg. of the nucleo-protein prepared from organisms grown in rat-serum broth. Tested 7 days later with living organisms grown in broth, only two of the rats died of plague. This survival rate is considerably higher than I have hitherto obtained by immunisation with material from broth-grown organisms (see this *Journal*, Vol. x. p. 559), so that one must conclude that the serum-grown organisms have lost none of their antigenic properties against broth-organisms.

It would appear from the above experiments that the extract from a race grown in serum provides a much more satisfactory immunising agent against a serum race of organisms than one prepared from broth-grown bacteria, and approaches the efficiency of vaccination with a living culture.

Vaccination with living attenuated cultures has been employed by Strong (1907) in Manila with satisfactory results—upwards of 200 persons having undergone this treatment. The cultures used by Strong remained stable during 18 months, and no unfavourable results were observed; nevertheless vaccination with a living culture has obvious disadvantages in its application to man. The whole subject of variation of bacteria has not yet been sufficiently studied to justify complete confidence that a race which has become attenuated by long cultivation upon artificial media in the laboratory might not, by growth in the body, unexpectedly recover some of its lost virulence. In the face of such a possibility, the question is not likely to be entertained by the authorities of India. If one understood precisely what happened in the process of immunisation by living culture, there is no reason to

<sup>1</sup> There are indications that organisms grown in the body possess a still higher degree of virulence which persists on cultivation in artificial media provided these contain serum proteins. The virulence of the test culture employed in this experiment was probably higher than that used in the previous experiments.



Fig. 1.



Fig. 2.



Fig. 3



Fig. 4.



suppose that the same result could not be brought about by the products of the plague bacillus or derivatives from them.

Immunisation, whether by dead or living cultures, is ultimately a question of the reaction of the tissues of the body to chemical stimuli. To obtain the optimum result it is necessary to arrange for suitable doses of the appropriate stimuli (i.e. the right antigen or antigens) and stimulation at the appropriate intervals. At present, this seems to be best brought about by the local propagation of an attenuated culture in the subcutaneous tissues of the animal. The bacilli growing in the tissue spaces provide by their lysis, and possibly also by their action upon the surrounding menstrea, the appropriate antigens, and it is not unlikely that the dosage and spacing of the stimuli may be found to be more favourable than the single injection of a quantity of an artificially prepared extract, even if this contained the same antigenic substances.

The aim I have had before me is to ascertain, and, if possible, imitate these conditions. The results detailed above indicate, I believe, an advance in this direction.

Throughout this work, much of which breaks unfamiliar ground, I have had the constant stimulant sympathy of Dr Charles J. Martin, Director of the Lister Institute. To him I offer my best thanks.

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LXV. THE INFLUENCE OF THE MEDIUM IN WHICH  
THE PLAGUE BACILLUS IS PROPAGATED UPON THE  
FACILITY WITH WHICH IT IS INGESTED BY HUMAN  
LEUCOCYTES.

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IN the following experiments various strains of plague bacilli grown in heated or fresh serum were used, and the opsonic technique followed was usually after the procedure of Leishman, that is to say, whole fresh blood was mixed with a bacillary emulsion in a capillary pipette, a drop of the resulting mixture placed on a slide and a cover glass immediately superimposed. The slide was then incubated for 15 minutes at 37° C. in a moistened Petri dish. At the end of this time the cover glass was drawn off, the films dried and then stained with dilute Giemsa stain.

The serum races were derived from, and the controls were made with, a strain of Bombay plague (B. 480) supplied me by Dr Rowland.

It has been demonstrated by Rowland (1913) that rats protected against a virulent broth-grown strain of plague, by means of previous injection of 0.1 mg. of nucleo-protein extracted from broth-grown organisms, have only slight protection against the same strain grown in horse serum (previously heated to 55° C.), or taken direct from the body of an animal infected by it. My earlier experiments were carried out to see if the strains grown in such heated serum were as readily phagocytosed by the leucocytes as the same virulent strain propagated on broth media.

All our laboratory strains are phagocytosed with avidity by human leucocytes. Löhlein (1906) found that a virulent laboratory culture of plague was freely ingested by the leucocytes of guinea-pigs and rats *in vitro*, but lost this property by "passage" through the guinea-pig. He attributed the resistance to phagocytosis to the development of a capsule, as had been found to be the case in the similar experiments of Grüber and Futaki (1906) with anthrax.

As the result of repeated experiments with heated horse serum strains, extending over many weeks, I failed to obtain any significant alteration in the extent to which phagocytosis occurred.

The following examples will bear out this point:

1. Equal volumes of fresh human blood and an emulsion made from a 48 hours' growth of B. 480 in heated horse serum.

Phagocytic index		Concentration of bacilli per c.c.
Heated serum race	Control, broth agar race	
6.47	6.93	$2000 \times 10^6$

2. *Wright's technique.*

Equal volumes of fresh human serum, human leucocytes and an emulsion made from a 24 hours' growth of B. 480 in heated horse serum.

Phagocytic index		Concentration of bacilli per c.c.
Heated serum race	Control, broth agar race	
7.05	7.43	$2000 \times 10^6$

3. *Wright's technique.*

Same as Ex. 2 but with rat serum and rat leucocytes.

Phagocytic index		Concentration of bacilli per c.c.
Heated serum race	Control, broth agar race	
4.97	6.23	$2000 \times 10^6$

Similar results were obtained by growing the bacilli in heated human, rat, or guinea-pig serum, that is to say, in no case was a significant variation obtained in the phagocytic indices with bacilli which had been grown in heated serum and on broth agar respectively.

I next proceeded to study the phagocytosis of bacilli procured from rats dead of laboratory plague infection. The pelvic gland of a rat which had just died of plague after ten days' infection was examined and the gland substance was found to be crowded with bacilli. An emulsion of the gland substance was made, then centrifuged at a low speed in order to deposit cellular elements and the supernatant fluid containing bacilli was used in the opsonic technique with my own blood. The slide on examination was found to be almost absolutely free from phagocytosed bacilli, the phagocytic index being only 0.12. As this experiment is open to objection on account of the possible presence of products of the plague bacillus in the material during the phagocytosis, I proceeded to test this result by isolating the organisms from the heart blood, growing them on agar, and using an emulsion in saline from this growth (48 hours) in the opsonic technique. A control experiment was also made with an emulsion of B. 480 which had been

grown without intermission on broth agar; the age of the culture used in this case was also 48 hours. A very striking diminution of the phagocytic index was observed in the former case. This experiment was repeated with the heart blood strain after successive subculture on broth agar on subsequent days and the following determinations were made:

*Phagocytic index of an organism obtained from the heart blood  
after successive subcultures upon broth agar.*

	(a) B. 480 passed through rat	(b) B. 480 control	Concentration of bacilli per c.c. employed
2nd day (1st subculture)	1.18	10.93	4000 million
4th day (2nd subculture)	1.35	19.46	6000 million
6th day (3rd subculture)	5.77	9.81	4000 million
7th day (4th subculture)	13.65	14.92	6000 million

It will be seen that by the fourth subculture within seven days after the death of the animal the phagocytic index was practically the same as with the control organism from which the animal was originally infected. The reduction in the phagocytic index which persists up to the fourth day is very striking.

It thus appears that the plague bacillus taken direct from an infected rat is little if at all ingested by human leucocytes, but by subculture outside the body upon broth agar during a period of about seven days the phagocytic index progressively increases till at last its value approximates to that of a strain long cultivated on broth agar.

This marked insusceptibility to phagocytosis observed when the plague bacillus is bred in the animal body, together with the fact that bacilli cultivated in heated serum are ingested as readily as those grown on broth agar, suggested that the increased resistance of the "Thierischen" strain might be referred to some property of fresh (unheated) serum. The following experiments (1 and 2) were therefore undertaken to investigate phagocytosis of the same strain (B. 480) of *B. pestis* as before when cultivated in fresh serum of the horse and rat respectively. A small sowing was used so that the resulting culture might be as free as possible from the original seed material.



*Experiment 1.*

·08 c.c. of a week-old culture of B. 480 in heated horse serum was dropped into 10 c.c. of absolutely fresh filtered horse serum and incubated for 18 hours at 37° C. The culture in fresh serum was centrifuged somewhat, to concentrate the scanty growth of organisms, and an emulsion of the strength of about 1000 millions per c.c. in serum was thereby obtained. An emulsion in saline of B. 480 (1000 million per c.c.) grown on broth agar was used as control.

	18 hours' culture. Phagocytic index	42 hours' culture. Phagocytic index	Concentration of bacilli per c.c.
Fresh horse serum race	0·08	0·56	1000 × 10 <sup>6</sup>
Control B. 480 on broth agar	5·13	—	1000 × 10 <sup>6</sup>

A loopful of the serum emulsion was transferred to a broth agar slope and incubated for three days, and an emulsion of 4000 million organisms per c.c. made therefrom. In this case the index rose to 13·00 as against a control of 13·31, showing how quickly the resistance to phagocytosis is lost after cultivation on ordinary laboratory media.

*Experiment 2.*

A similar experiment was performed with a culture on blood agar from a rat infected with the strain B. 480, the organism was transferred from blood agar and grown for 18 hours in fresh filtered rat serum.

Strain	Phagocytic index	Concentration of bacilli per c.c.
A. Final rat serum strain	0·36	2000 × 10 <sup>6</sup>
B. Control B. 480	7·85	2000 × 10 <sup>6</sup>
C. Emulsion from agar culture inoculated from intermediate blood agar culture at same time as "A."	8·52	2000 × 10 <sup>6</sup>

Again the same result is seen. A striking difference in the phagocytic index is produced in a very short time by growing the same strain of organisms, in the one case in fresh rat serum, and in the other on broth agar, the value of the index being more than twenty times greater with the broth agar organisms.

A broth agar growth was prepared from this resistant strain "A" and incubated for three days at 37° C. As is seen below, the resistance to phagocytosis was thereby lost.

Strain	Phagocytic index	Concentration of bacilli per c.c.
Agar slope culture from fresh rat serum race "A"	11·97	4000 × 10 <sup>6</sup>
Control B. 480	13·31	4000 × 10 <sup>6</sup>

The following experiments were undertaken to ascertain whether the resistance to phagocytosis possessed by organisms recently removed from the body and propagated on blood media is a property of the bacillus or due to the action of some toxic product upon the leucocytes.

Ten c.c. of filtered fresh rat serum were inoculated with four drops of a strain derived from a growth from heart blood on blood agar. On the following morning the growth was centrifuged down, and phagocytic determinations were made with the following mixtures:

1. Centrifuged deposit of organisms and bottom layer of serum.
2. Upper layer of serum free from organisms+emulsion of B. 480 grown on broth agar.
3. Saline emulsion of culture of B. 480, grown on broth agar.
4. Same as Experiment 1 but with addition of emulsion of *Staphylococcus pyogenes albus*.

All emulsions contained 2000 million plague bacilli per c.c. and were mixed with equal quantities of fresh human blood.

The figures obtained were as follows:

Experiment	Phagocytic index
1	0.71
2	5.55
3	5.90
4	Marked phagocytosis of cocci

The results of Experiments 2, 3 and 4 show that the low index obtained in Experiment 1 is not to be explained by inhibition of leucocytic action due to the presence during phagocytosis of any substance derived from the medium (rat serum) in which the bacilli had been grown and in which the bacilli were emulsified for the test. Under exactly similar conditions of experiment, normal phagocytic indices were obtained both with *Staphylococcus pyogenes albus* (Exp. 4) and with a control culture of plague bacilli grown on broth agar (Exp. 2). The low index obtained in Experiment 1 must therefore be attributed to special properties of resistance inherent in the race of bacilli grown in the fresh serum.

The resistance to phagocytosis of organisms taken direct from the body of an infected animal is not unexpected, as bacilli are rarely seen inside leucocytes in acute plague. It is also possible to endow bacilli long propagated upon laboratory media with this characteristic by educating them to grow in quite fresh serum of the rat.

It is, however, remarkable that growth in *heated* serum should yield bacilli whose resistance to phagocytosis is in no way enhanced, but remains the same as that of the original broth agar race, for in the experiments of Rowland (1913) virulence was depressed by growth in fresh serum and heightened by cultivation in heated serum.

This result would suggest that virulence is not necessarily correlated with resistance to phagocytosis.

SUMMARY.

1. An old laboratory strain of *B. pestis* grown in heated horse, rat, guinea-pig or human serum, did not show any appreciable variation in the phagocytic index with human leucocytes when compared with the original culture.

2. Strains of plague bacilli taken direct from an animal dying of the disease show a marked reduction in capacity for phagocytosis, which persists for a short time after cultivation on broth agar outside the body.

3. Laboratory races of plague bacilli grown in fresh rat or horse serum show a high resistance to phagocytosis and are not ingested by human leucocytes to any appreciable extent.

4. Since, as shown by Rowland (1913), virulence of *B. pestis* is enhanced or depressed according as the organism is cultivated in heated or fresh serum respectively, power to infect would appear to depend upon factors other than resistance to phagocytosis.

I wish here to acknowledge my indebtedness to Dr C. J. Martin, F.R.S., Director of the Lister Institute, and to Dr Sydney Rowland of the Indian Plague Commission for much valuable advice and suggestions throughout this enquiry.

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## LXVI. THE MORPHOLOGY OF THE PLAGUE BACILLUS.

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(With Plates XVII—XXIII.)

DURING the progress of my work upon plague vaccines it has been necessary to grow the bacillus upon various media under a variety of conditions, and some of the instances of pleomorphism of this organism seemed worthy of being placed upon record.

Probably no organism presents so marked a pleomorphism as the bacillus of plague. A glance at the photomicrograms which accompany this report, Plates XVII—XXIII, will demonstrate the truth of this statement. We find amongst them forms simulating micrococci, streptococci, bacteria, streptothriciae and even mould forms. The illustrations are from specimens either fixed and stained or from living specimens of the bacillus.

The dark ground illumination which was used to photograph some of the pictures was obtained by a central stop in an aplanatic condenser. By this means, a low-angled illuminating cone is produced in contrast to the excessively high angle of the rays emerging from a paraboloid. As has been pointed out already in these reports, this is a decided advantage as it eliminates light-haze from small or ultra-microscopic particles (see previous report, *Journal of Hygiene*, XII, p. 362).

If a broth culture of plague be examined by transmitted light it will be found that around a very small proportion of the bacilli a delicate halo can be observed. This is extremely faint and is visible in only a small proportion of the organisms. It is best seen when the edge or boundary line of the bacillus proper is in focus. So faint is it that unless some confirmatory evidence of its presence be forthcoming reliance could hardly be placed on the appearance as evidence of a definite structure.

If the culture be centrifuged, the bacilli washed in water, again centrifuged and taken up in a drop of Indian ink the hitherto faint aureole surrounding certain of the bacilli becomes strikingly visible.

Some idea of the appearance presented is given by the photomicrograms (figs. 18, 19 and 20) in Plate XXI.

The finest particles of ink impart a grey background to the picture and the larger particles show up black; all are in rapid Brownian movement, being small enough to respond to molecular bombardment. The limiting membrane of the bacillus can be sharply focused, and between it and the general black or dark grey background is a perfectly clear area free of all particles; the width of this area is often greater than the diameter of the bacillus. At the edge of this layer the Brownian movement is intense, the appearance suggesting that the particles of ink are prevented from bombarding the bacillus by some invisible envelope.

This envelope is a definite entity. It recalls the capsules of certain bacteria and more especially the slimy shell of *Bacillus tumescens*. Plague bacilli occasionally possess true capsules but the appearance I am describing differs from a capsule in having no definite outer limit. A typical capsule such as that possessed by the *Pneumococcus* is easily seen in an unstained specimen by reason of its sharp outer edge and its high refractive index. The layer around these plague bacilli is hardly visible in an unstained specimen mounted in a clear fluid and as seen in the ink preparation it possesses no sharply defined outer edge. If the cover-glass be tapped judiciously with a needle the layer is seen to possess little rigidity and, by lucky manipulation of the needle, can be drawn out into a streaming appendage resembling the tail of a comet. Its consistency is judged to be viscid. This observation reminds us of the well-known stickiness of plague cultures on solid media.

The envelope is insoluble in water but readily soluble in dilute alkalis.

To obtain nice preparations the bacilli should be centrifuged and washed in water. This is especially necessary when examining cultures growing in serum or serum broth, as Indian ink possesses to an extraordinary degree the property of adsorbing proteins, after which the particles no longer remain discrete, but flocculate and fail to afford the necessary Brownian movement to show the envelope clearly. Preparations made as above and sealed with paraffin remain for days unaltered.

The medium in which the bacilli are propagated exerts an influence upon the development of the envelope.

If the presence of the envelope be compared in two cultures, one grown in broth, the other grown in broth containing 10% serum (previously heated to 55° C. for half an hour), the number of organisms with a well-marked envelope is seen to be enormously increased in the latter case. In a broth culture it is present only in a minority of the organisms;

in the serum-broth culture practically every bacillus or chain of bacilli possesses it. In the case of chains, which are usual in serum-broth cultures, one chain is embedded in a common envelope.

The temperature at which the culture is incubated has also considerable influence on the presence of the envelope.

Thus a broth culture propagated at 20° C. presents organisms all of which are naked, whereas in the case of propagation at 36° C. about 50 % of them are clothed with this envelope.

The envelope is also well marked in those organisms which I have examined, taken straight from the spleen of a rat dead of plague.

If a serum-broth culture be prepared and the organisms washed and transferred to perfectly fresh normal rat serum or immune horse serum and incubated at 37° C. for half an hour the envelopes that were practically universal in the case of the serum-broth culture are found to have disappeared almost entirely.

Under certain conditions the plague bacillus develops a capsule which has a sharp, regular outline; which can be distinctly seen in unstained specimens and is coloured by the usual capsule stains. This structure has been described by several observers; Kitasato (1894), Yersin (1897), Zettnow (1896), Albrecht and Ghon (1900), Wherry (1905), and Lohlein (1906), both in the body and culture media. All observers agree, however, that the presence of capsules is inconstant and generally difficult to demonstrate. From the descriptions and figures given I have no doubt that in many cases the authors were dealing with the slimy envelope I have described above. There are, however, often intermediate appearances when it is difficult to decide whether capsules exist or not.

I have only met with a definite capsule in bacilli:

- (1) At the site of inoculation in experimentally infected rats.
- (2) In bacilli that have been grown in a serum medium.

Some normal sera, notably that of the horse, have the property of lysing certain strains of living bacilli. Under these circumstances I have sometimes noticed that many of the bacilli develop a definite capsule which is plainly visible under dark ground illumination and can be stained with the usual capsule stains.

The following technical data refer to the series of microphotograms illustrating the pleomorphism of the plague bacillus, and the existence in some cases of a layer of viscid material surrounding the bacilli.

A. Stained preparations.

Magnification. 1000 diameters.

Illuminant. Open arc 4 amps. D.C.

Condenser. Zeiss aplanatic.

Objective. 2 mm. 1, 40, 10" tube.

Ocular. Projection No. 4.

Screen. Wratten and Wainwright B screen transmitting light from 6000 A.U. to 4600 A.U. with screen G transmitting from red end to 5100. The two screens combined transmit a monochromatic band from 5100 to 6000 A.U.

Stain. Carbol thionine. This stain has a strong absorption band extending from 5500 to 6800.

B. Dark ground preparations.

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### DESCRIPTION OF PLATES XVII—XXIII.

(Magnification 1000 diameters.)

- Figs. 1 and 2. Stained preparations from growth on agar and in broth. The bacillus stains for the most part uniformly.
- Figs. 3 and 4. Growth in broth to which had been added 10 % horse serum previously heated to 55° C. for half an hour. Growth in chains with well marked bi-polarity.
- Figs. 5 and 6. Growth in the spleen of a rat dead of pest. Note the pseudo-capsules (envelopes).
- Fig. 7. Smear preparation from spleen of plague infected rat showing pseudo-capsules (envelopes).

## 422      *The Morphology of the Plague Bacillus*

Figs. 8, 9, 10 and 11. Mould forms.

Fig. 8. From an old abscess at the seat of inoculation in a rat dead of pest.

Figs. 9 and 10. From an old growth in serum broth. Living specimens photographed with dark ground illumination.

Fig. 11. From an old abscess at seat of inoculation.

Figs. 12 and 13. Pfaundler's Balls, a form observed when growth takes place in immune serum.

Figs. 14 and 15. Another form of growth occasionally observed in rat serum that has been heated to 55° C. for half an hour.

Figs. 16 and 17. Yeast-like forms often observed at the site of inoculation in rats.

Figs. 18 and 19. The plague bacillus, 18 hours' culture in 10 % serum broth, as seen in unstained specimens by the aid of the ink process. The organisms were centrifuged down, washed in distilled water, again centrifuged and the residue taken up in Indian ink, a sufficiently fine layer for examination being obtained by pressing a flat cover-glass on a flat slide with a drop of the ink emulsion between.

Fig. 20. A specimen similarly prepared to those illustrated in Figs. 18 and 19. In this case the centrifuging has been more energetic, forcing several previously discrete organisms provided with envelopes into fusion.

Figs. 21, 22, 23, 24, 25 and 26. Involution forms seen when the plague bacillus is grown in broth containing 2 % sodium chloride. Two days growth. Photographed unstained and alive with dark ground illumination. These well-known involution forms are very difficult to stain as they contain so much water in the bladder-like swellings that fixing is almost impossible.

Figs. 27, 28 and 29. A short chain growing in fresh horse serum. The chain is surrounded with a well-marked capsule. MacConkey's capsule stain was used for these and the following two figures.

Fig. 30. Three short chains lying close together. In the lowest chain one individual appears normal, in the remainder the bacillus is undergoing lysis and of the chromatin a few dots alone remain.

Fig. 31. Two individuals. One unprovided with a capsule the other capsulated and the chromatin reduced to two dots.

Figs. 32, 33, 34, 35 and 36. Living bacilli from a culture in spleen juice showing well developed envelopes surrounding each chain. The preparation was made by the Indian ink method (see description to Figs. 18 and 19 above) but in this case illuminated by means of a paraboloid condenser. The ground between the bacilli and outside the enveloping layer is bright as the particles of ink scatter the light.

Figs. 37 and 38. The wet preparation from which Fig. 36 was made was ringed round with gold size. When this was dry a small hole was made in the ring of size which cemented the coverslip to the slide. This allowed of a very slow desiccation of the preparation. The preparation was dry in 6 days. This was evident by the cessation of the Brownian movement in the preparation and by the adhesion of the ink particles to the coverslip. The coverslip was then carefully lifted so as to disturb the film of dry ink and bacilli as little as possible. The film was then stained with MacConkey's capsule stain. The picture shows that the bacilli alone take the stain, the layer remaining colourless. Compare these figures with Figs. 27, 28 and 29 above showing the true capsules which stain well.





Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



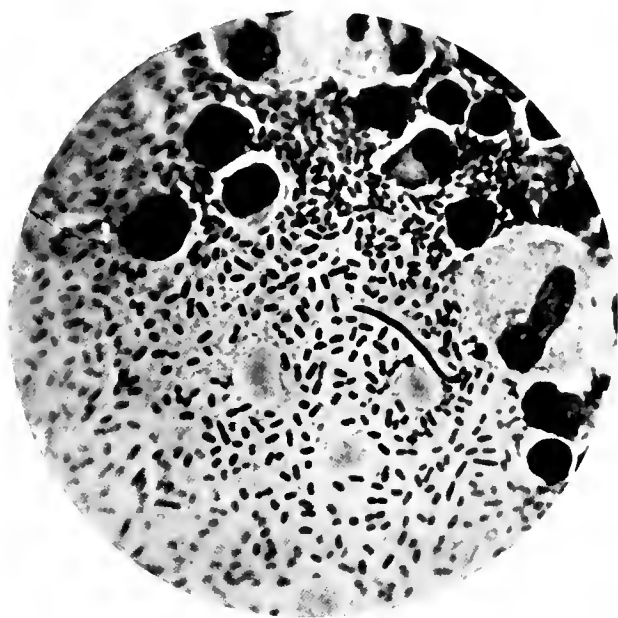


Fig. 5.

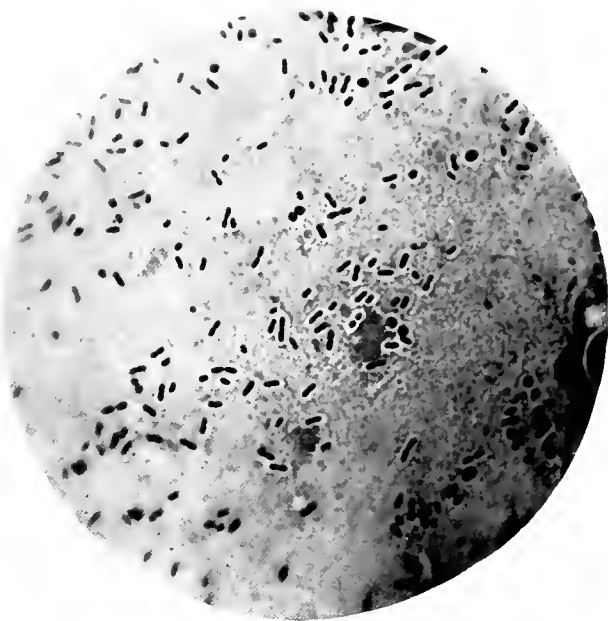


Fig. 6.

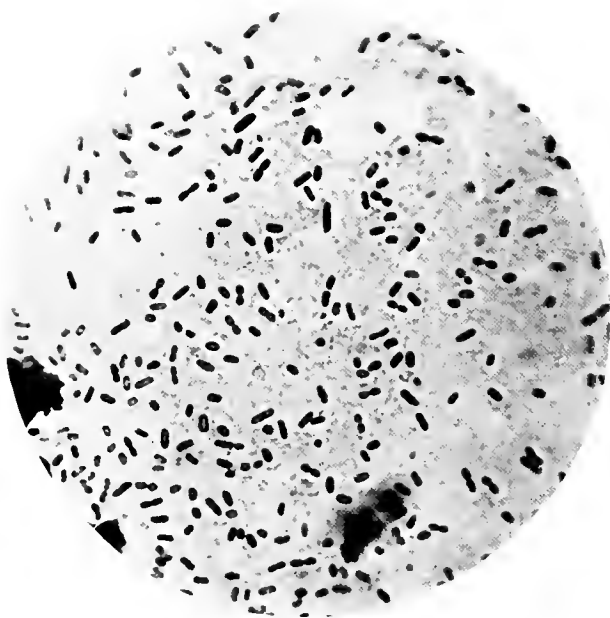


Fig. 7.



Fig. 8.





Fig. 9.



Fig. 10.



Fig. 11.

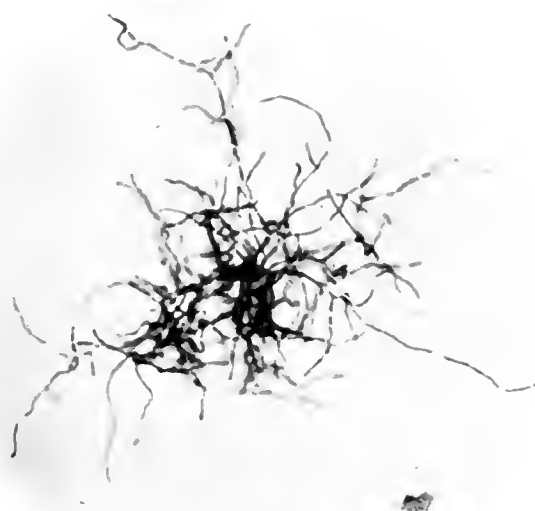
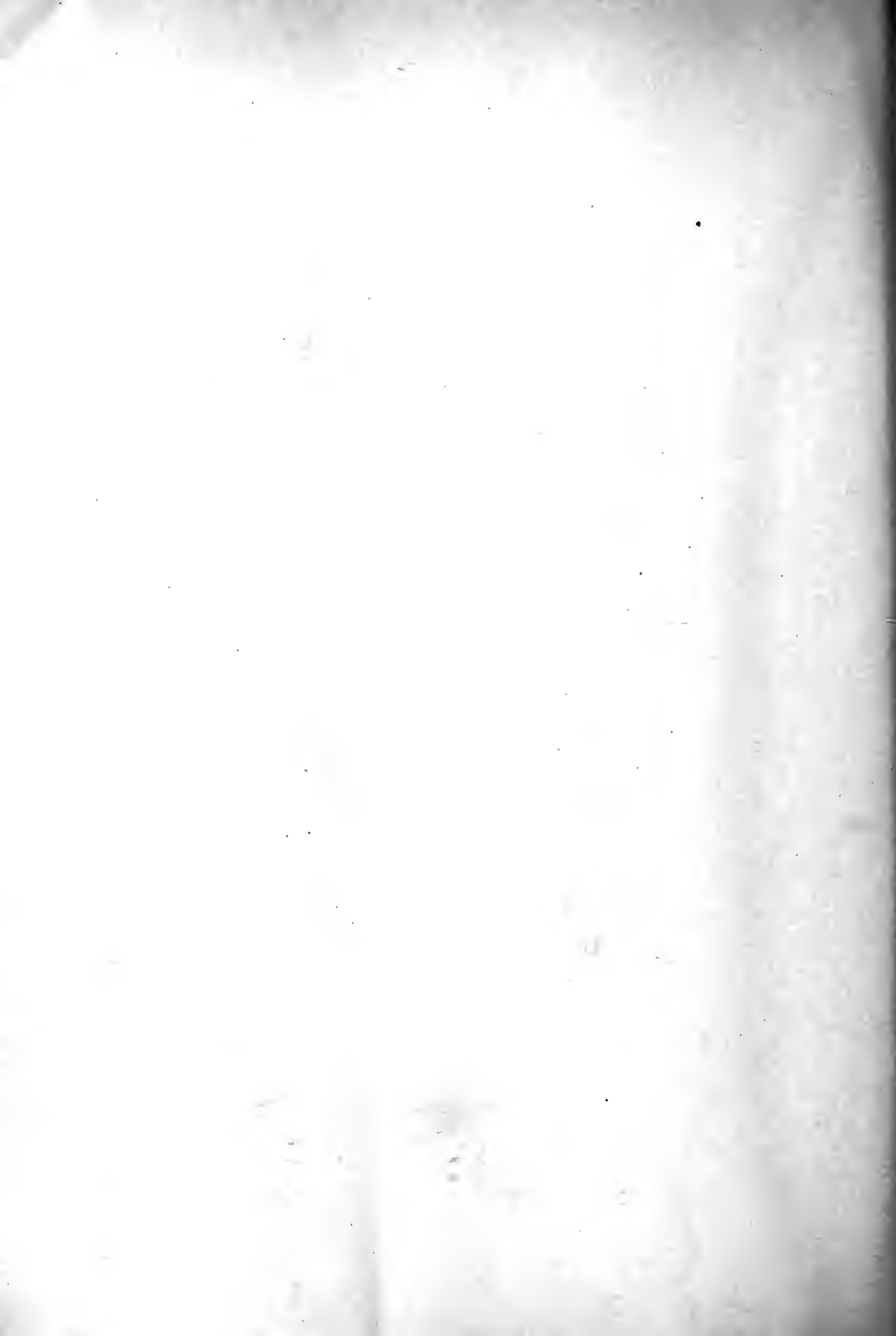


Fig. 12.



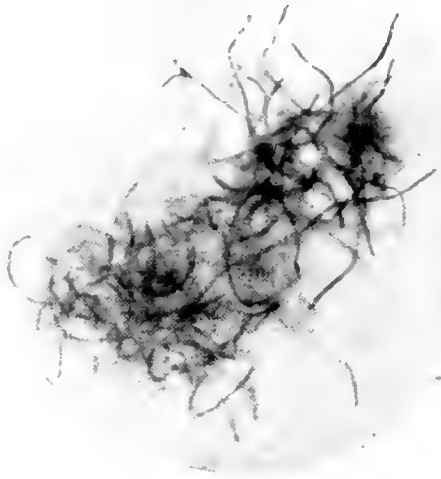


Fig. 13.



Fig. 14.



Fig. 15.

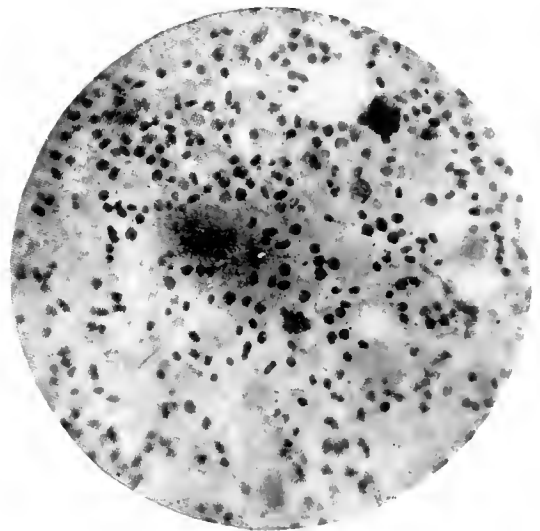


Fig. 16.





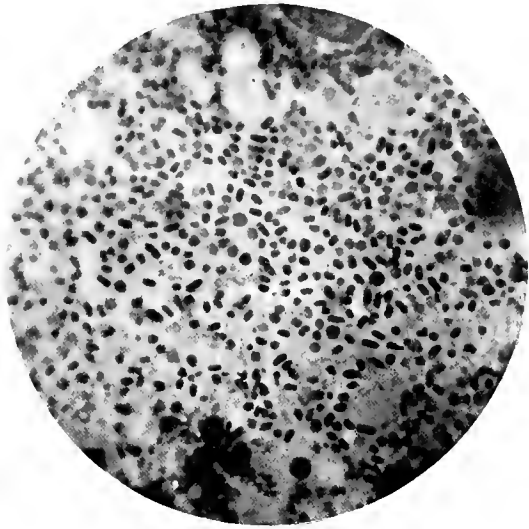


Fig. 17.

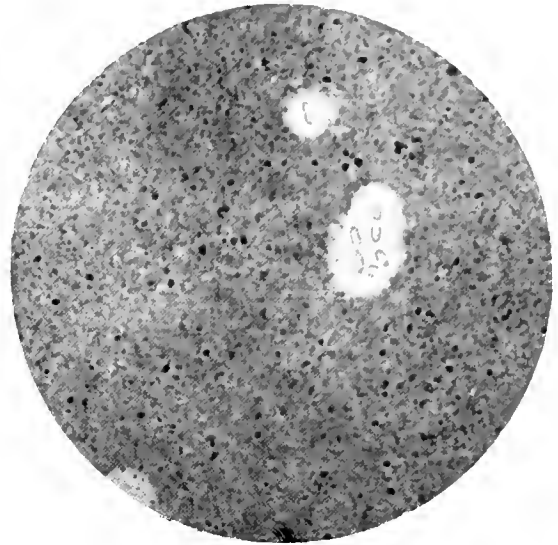


Fig. 18.



Fig. 19.



Fig. 20.

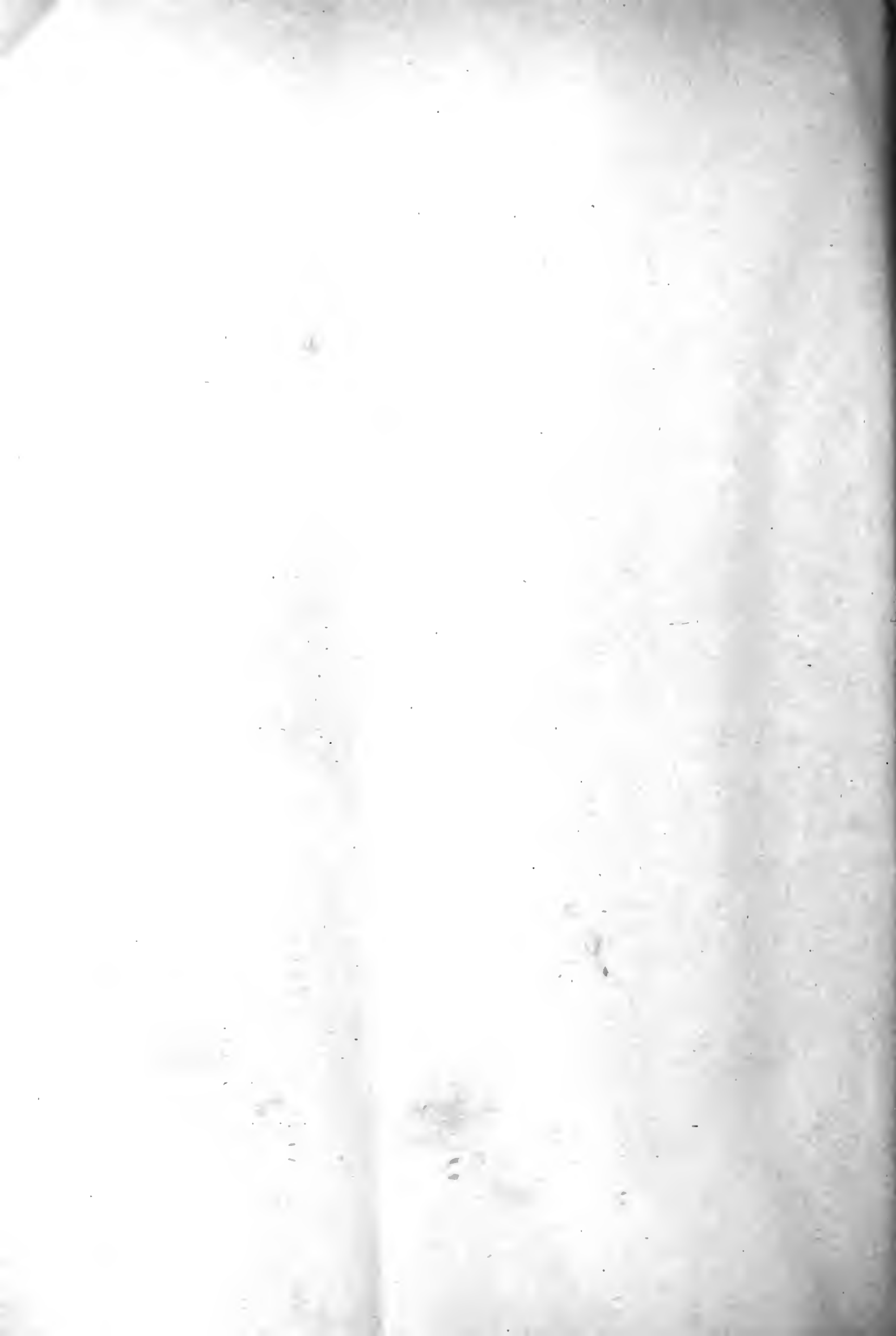




Fig. 21.



Fig. 22.



Fig. 23.



Fig. 24.



Fig. 25.



Fig. 26.





Fig. 27.



Fig. 28.



Fig. 29.



Fig. 30.



Fig. 31.



Fig. 32.



Fig. 33.



Fig. 34.



Fig. 35.

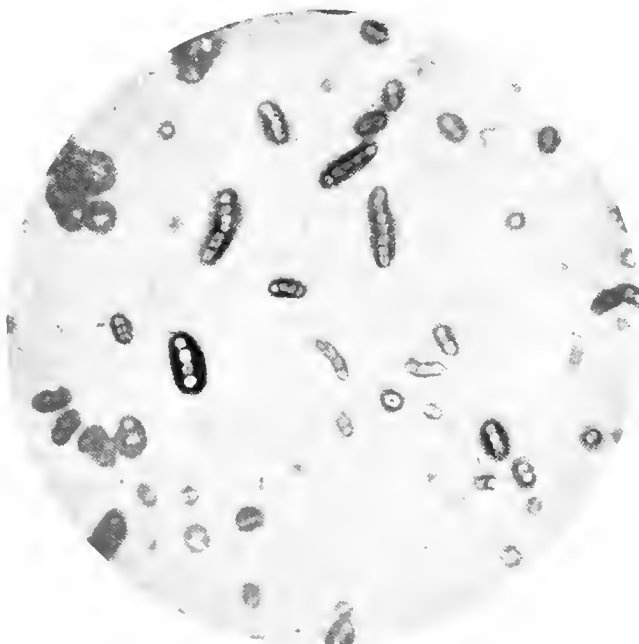


Fig. 36.



Fig. 37.



Fig. 38.



## LXVII. OBSERVATIONS ON THE MECHANISM OF THE TRANSMISSION OF PLAGUE BY FLEAS.

BY A. W. BACOT, *Entomologist, Lister Institute*,  
AND C. J. MARTIN, *Director, Lister Institute*.

(With Plates XXIV—XXVI and 4 Text-figures.)

THE literature on the transmission of plague by fleas was reviewed in 1905 in the first series of Reports on Plague Investigation in India (*Journal of Hygiene*, Vol. VI. p. 422), and it is only necessary to epitomise briefly the main facts here. The conclusion, that this insect plays an important rôle in the spread of plague, was arrived at on epidemiological grounds by Ogata (1897), Simond (1898), Ashburton Thompson (1903), and Liston (1905). Simond also made a few experiments, which strongly indicated that infection from rat to rat could be brought about by the agency of fleas. Gauthier and Raybaud (1902 and 1903), and Verjbitzki (1904) by more extensive, more varied and better controlled experiments, confirmed Simond's observations, and proved clearly the possibility of transmission by this agency.

The question of flea transmission and its epidemiological importance was extensively studied by the Commission for the Investigation of Plague in India (1906 and 1907). They found that, once control of the experimental conditions had been obtained, transmission from one animal to another could readily be brought about by fleas, and further made the very important observation that only in the presence of fleas did an epizootic amongst rats or guinea-pigs ensue. Close contact with infected animals, including the devouring of infected carcasses, was occasionally followed by a case of plague, but no spread occurred.

A number of experiments, in which animals were allowed to remain in animal houses in which epizootics had occurred and in plague-infected native quarters, resulted in infection, but if the simplest measures were taken to prevent the access of fleas to animals, they remained unaffected—showing that the infection lurking in such situations was resident in the flea population.

Simond was of opinion that infection was caused by the rat rubbing flea-faeces containing plague bacilli into recent flea-bites.

Verjbitzki (1904) demonstrated that the puncture in the skin occasioned by the bite of bugs and fleas affords a channel through which plague bacilli can enter, for the application of crushed infected bugs and fleas and their faeces, as well as other plague material to the situation recently bitten, was found to infect an animal; more than one puncture was requisite to obtain infection.

The Commission for the Investigation of Plague in India (1907), 2nd Plague Report, discusses the following possible methods by which the flea may transmit plague:

1. By the animals eating the infected fleas.
2. By the proboscis of the flea mechanically conveying the bacilli from the infected to the healthy animal.
3. By the salivary glands of the flea becoming infected, the bacilli being then inoculated along with the saliva.
4. By a regurgitation of the stomach contents through the oesophagus and pharynx, the bacilli being then injected with the saliva, or on the pricker, or being rubbed into the wounds made by the pricker.
5. By a retention of infected blood in the pharynx or about the mouth-parts of the flea, the bacilli multiplying there and then being inoculated into the animal in the same manner as in hypothesis No. 4.
6. By the bacilli contained in the faeces being deposited on the skin, and then being either injected by the pricker or rubbed into wounds made by the pricker.

Methods 1 to 3 are set aside on what seem to us satisfactory grounds, No evidence could be found for 4 or 5. It was, however, shown that infection could be brought about by smearing recent flea-bites with septicaemic blood or a virulent culture of plague, and the conclusion is arrived at that the possibility of infection by the rat rubbing flea-faeces into recent flea-bites is demonstrated, but on the question whether this is the usual method of infection, the Commission did not feel justified in expressing an opinion.

The Commission dissected many hundreds of fleas and searched for the presence of plague bacilli in their salivary glands and body cavities, but on no occasion, either by microscopic examination or by culture, were they able to find plague bacilli outside the alimentary canal of the insects. We may say at once that our own observations on this point coincide with those of the Commission, and it appears certain that transmission is not occasioned through infection of the salivary glands of the insect transmitter, as in the case of malaria and sleeping sickness.



The probability of an infection taking place by the inoculation of infected flea-faeces into flea-bites has been questioned by Cranston Walker (1911). This observer experimented upon himself and others—using tuberculin, vaccine lymph and a virulent culture of *Staphylococcus albus*. Having found that the application of each of these materials, either before or after the skin was punctured with a fine needle, .37 mm. in diameter, gave upwards of 90% successful inoculations, he repeated the experiment with the puncture made in each case by one flea. The diameter of the proboscis of this insect is about .02 mm. Only with the tuberculin did the flea puncture succeed in producing an inoculation, and then only in a small minority of cases. Walker's observations show that, although one hole .02 mm. in diameter does not usually admit of sufficient tuberculin, vaccine lymph or staphylococci to occasion infection, a hole 20 times as large does. To produce infection the number of plague bacilli necessary to be inoculated is, however, probably much less than in the case of staphylococci. As shown by Barber (1912) *one* may suffice. Moreover, under natural conditions, whether in rat or man, scratching is not uncommon after flea-bites, whereas this was avoided in Walker's experiments. We do not think these observations very seriously militate against the view that infection of plague may be brought about by rubbing flea-faeces containing plague bacilli into recent flea-bites, with the probable assistance of scratching.

We have repeated the experiments of the Commission on this point, applying to the bitten area (*a*) the surface of the spleen of a rat recently dead from plague; (*b*) a strong emulsion of plague bacilli from the stomachs of fleas which had been nourished on animals with septicæmia. The stomachs used were full of plague culture (see p. 435 below).

The experiments were performed as follows:

A number of rats were carefully shaved over a part of the abdomen a few square cms. in area. Three days later 20 normal fleas were given the opportunity to feed upon the rats. The fleas were in a test-tube, the mouth of which was covered with gauze, and the mouth of the tube was applied to the shaved area for about one minute. The majority appeared to feed, but as many of the insects retire to the lip of the test-tube for the purpose, they could not all be seen. This manœuvre on their part results in a ring of punctures round the circumference of the test-tube. Immediately afterwards, in Series A, the cut surface of a spleen was gently applied to the same area and, in Series B, the

emulsion was dabbed over it with a pledget of cotton-wool. The rats were immediately returned to separate cages.

A third control series, Series C, contained 13 rats, which were treated in the same manner as those of A, with the omission of the flea-punctures.

Most of the rats promptly licked off the spleen-pulp or emulsion which had been applied.

Series A consisted of ten rats, of which nine died of plague in under 3 days.

Series B consisted of 23 rats, of which five died of plague.

Series C consisted of 13 rats, none of these contracted the disease.

Four out of the nine rats of Series A which contracted the disease displayed phlebotomies—in two cases arranged in a ring corresponding to the situation of the punctures. All showed buboes either in the axillae or groin.

The experiments show a striking difference in the incidence of infection. This might be accounted for either by variation in the number of bacilli deposited or in their virulence. We incline to the latter interpretation, as we endeavoured to arrange that the amount of bacilli applied should be of the same order. We have not actually determined the relative virulence of the two kinds of organisms, as this could only be done by experiments on a long series of animals. One significant difference in the properties of the two strains of organisms was, however, observed. When mixed with a drop of our own blood, and incubated for 15 minutes between a slide and cover glass, as in the original method devised by Leishman for the quantitative estimation of phagocytosis, the bacilli direct from the spleen were not taken up by the phagocytes, whereas those from the flea's stomach were freely ingested.

The experiments were performed as follows: to obtain bacilli from the spleen a piece of the organ was minced up with .85% NaCl solution and centrifuged at a low speed until the tissue cells and blood corpuscles were deposited. After centrifugation the plague bacilli were partly in suspension and partly in a loose deposit on the surface of the cells. This loose deposit was dispersed into the medium by gently shaking, without disturbing the cells, etc., and pipetted off. The emulsion was centrifuged at a high speed, the supernatant fluid, which now consisted of bacilli with an occasional cell, removed, and the deposit emulsified in fresh saline. The emulsion consisted only of single bacilli. The number of bacilli per c.c. were determined by counting in a Thoma Zeiss cell,

using dark-ground illumination. The emulsions were diluted so as to contain approximately 1000 million bacilli per c.c.

To obtain an emulsion of the bacilli from the flea's stomach the contents of 30-40 strongly infected stomachs (see p. 435 below) were rubbed up with saline by means of a short stumpy brush made of cotton wool, centrifuged at high speed, and the supernatant fluid removed. The deposit was re-emulsified in the same manner and centrifuged at low speed to get rid of the aggregates. The emulsion was not good and still contained many clumps of bacilli, but from the nature of the material this could not be overcome. The bacillary content of the emulsion was counted in the way mentioned above. The emulsion was subsequently diluted so as to afford about 100 million per c.c. A stronger emulsion could not be made, as the amount of material was inadequate.

One volume of each of these emulsions was mixed with one volume of the blood of one or other of us, and a suitably sized drop placed upon a glass slide, covered and incubated for 15 minutes at 37° C. in a saturated atmosphere. The coverslip was slid off the slide, and as soon as dry both films were fixed in alcohol and stained with Leishman's stain.

The experiment with both varieties of bacillus was made on three occasions; on each occasion the appearance presented on examination of the two sets of films was strikingly different. Whereas the majority of the polymorphonuclear leucocytes had taken up bacilli derived from the fleas' stomachs, the average number per leucocyte varying from three to five in the different experiments, we found none of the bacilli taken directly from the spleen inside the cells.

These observations in conjunction with the lesser infectivity of the bacilli from the fleas' stomachs lead us to conclude that by growth in the stomach of the insect, a race of diminished virulence had bred out, which had lost the resistance to phagocytosis possessed by the original blood-strain. In an adjoining paper in this *Journal* by St John Brooks (1913) the author shows that this property of blood-strains of plague is rapidly lost by a few generations on broth agar. The blood in the stomach of the flea is soon attacked by the digestive ferments, so that it is likely that the characteristics of the medium are soon lost and that the bacilli are really nourished on the hydration products of protein. The digestion is an alkaline one.

*Observations showing that infection may occur during  
the act of sucking.*

There is no doubt plague may be inoculated by the dejecta from infected fleas. In the case of a flea-infested rat, the hair, especially in those areas where the animal cannot dislodge them, such as the back of the neck and root of the tail, is often sprinkled with the dried excrement from the parasites, and the animal is frequently to be seen scratching itself. Nevertheless, we have long felt dubious as to whether this was the only, or even the principal method by which infection is conveyed. In comparison with the masses in the fleas' stomachs, the faeces do not as a rule contain many bacilli, and soon dry up. We have also given the reasons which lead us to believe that bacilli which have grown in the stomach of the insect are not of a high degree of virulence. Infection by this means must leave much to chance.

We therefore set out to ascertain whether or not the flea could infect during the act of sucking. The Commission for Investigation of Plague in India frequently conveyed plague to guinea-pigs by feeding infected fleas through fine muslin gauze. The same was done by Swellengrebel (1913) in Java. This suggests that infection at the time of feeding may not be an unusual occurrence, but the Indian observers were not satisfied that by this means inoculation by faeces was excluded, for the insects defaecated on the muslin at the time of the experiment.

METHODS OF EXPERIMENT.

In our experiments, we fed under supervision on rats infected fleas which had been deprived of food for from 24 to 28 hours. Each flea was watched with a hand-lens during the act of sucking and removed to a test-tube directly it had completed its meal. Such starved fleas rarely pass faeces at the time of feeding, and, in the very few instances when this occurred, the dejectum was removed by the corner of a piece of blotting-paper and strong lysol immediately applied to the spot. Under the conditions of our experiments inoculation of possibly infected faeces was excluded. The fleas belonged to the species *Ceratophyllus fasciatus* and *Xenopsylla cheopis*.

Our first care was to obtain a supply of the fleas well infected with plague. White rats, although quite susceptible to plague, usually die before a high degree of septicaemia has developed. As, to obtain a satisfactory supply of infected fleas, numerous microbes in the blood of

the host are necessary, our first attempts were not very successful. Subsequently, this difficulty was overcome by feeding our fleas on mice.

Mice are not so susceptible to plague as rats, but the degree of septicaemia intervening before the death of the animal is often extraordinary. In one case we counted 2·7 bacilli for every corpuscle (Plate XXIV, fig. 1) and the number of organisms frequently equalled that of the blood corpuscles. Neither rat-flea feeds upon mice with the same readiness as upon rats, but as no other item in the *menu* was provided, they fed well enough for the majority of our insects to become infected.

The fleas were kept in a glass cage with several inches of sawdust, and containing two separate compartments screened off by coarse meshed wire gauze for the mice. The cages were similar to those employed by the Indian Commission for their transmission experiments (1906), and are shown in Plate XXIV, fig. 2. The cages were supported above a shallow tray containing lysol, which extended six inches beyond their margin. At first, the mice were inoculated with plague, and replaced by fresh inoculated mice every two days as they died. As time went on it was not found necessary to supply inoculated mice, as normal mice became infected from the fleas with regularity. The fleas bred in the cages and the system became automatic—the fleas infecting the mice, and these in turn infecting other fleas.

To collect a supply of fleas for an experiment some of the sawdust in a cage was decanted into a wide bowl, commonly known as a "chef-bowl," 17 inches in diameter, with smooth white surface and nearly vertical sides of 9 inches. As the fleas emerge from the sawdust on to the white surface they are readily seen and swept into test-tubes by a paint brush. A small loose fragment of cotton-wool was added for the insect to perch in.

The rats were carefully shaved three days previous to use, so as to avoid any minute abrasions. In order to immobilise them at the time of feeding we employed the method used by Chick and Martin (1911). The rats were gently bandaged with soft gauze bandage, with all four legs in the extended position, but leaving the abdomen exposed. When this is done, the animal seems quite comfortable and remains quiet when laid on its back; although it could quite easily disengage itself, it does not seem to know how to begin. A white rat so secured will lie supine for an hour or more. Each animal was placed upon a pad of cotton-wool, about 18 inches square. Any flea which jumped landed in the cotton-wool and became entangled.

To feed the fleas, the test-tube was inverted over the shaved area,

and, as soon as the insect had settled down to feed, the test-tube was removed and it was watched with a hand-lens. If the fleas are allowed to reach the skin of the rat through a minute and loose fragment of cotton-wool, or to burrow in cotton-wool before being placed on the shaved area, they will feed more readily. The wool possibly suggests fur to them and they feel more at home. Before each flea had filled its stomach it was removed by the leg with a fine pair of forceps and transferred to its test-tube, but in Series III, IV and V fleas were usually removed by entangling in cotton-wool to avoid any injury to their limbs. In the experiments of Series I and II they were subsequently dissected, and a film made of the contents of their stomachs to ascertain whether they were infected with plague bacilli.

Our cages were stocked with a supply of fleas in January of this year, and evidence of the fleas being infected and capable of transmitting plague to the uninoculated mice was forthcoming nine days later.

#### SERIES I.

Our first experiments were made with fleas of the species *Ceratophyllus fasciatus*, before the population was strongly infected. A number of fleas, varying from 7 to 27, were separately fed for one minute upon a rat. 25% of the fleas were afterwards found to contain plague bacilli in their stomachs.

None of the animals contracted plague. The protocols of the experiment are given in Table I below:

TABLE I. *No. of rats in the series, 10.*

Min. No. of fleas fed on 1 rat ...	7
Max.     "     "     "     " ...	27
Mean     "     "     "     " ...	16
Min. No. infected fleas fed on 1 rat ...	2
Max.     "     "     "     " ...	7
Mean     "     "     "     " ...	4
Mortality from Plague, 0.	

#### SERIES II.

The second series of experiments was made a week later when the insects were more heavily infected. *Ceratophyllus fasciatus* was the species of flea used, and the experiments were conducted in a similar way to those of the previous series. The proportion of infected fleas

was, however, much greater, 60% of those used being subsequently found to contain plague bacilli. The degree of infection of the individual insects was also higher. The details are summarised in

TABLE II. *No. of rats in the series, 10.*

Min. No. of fleas fed on 1 rat ...	15
Max.       ,,       ,,       ,, ...	26
Mean       ,,       ,,       ,, ...	20
Min. No. infected fleas fed on 1 rat ...	5
Max.       ,,       ,,       ,, ...	25
Mean       ,,       ,,       ,, ...	12.5
Mortality from Plague, 2.	

These experiments show that infection may be conveyed during the act of feeding, but that it by no means occurs every time a flea with plague bacilli in its stomach feeds on a susceptible animal.

### SERIES III.

The third series of experiments was made with what we designate "fleas certified as plague infected." A number of fleas were segregated and the faeces deposited examined daily for plague bacilli, and only those passing bacilli were used for the experiment. Twenty "certified" fleas were given the opportunity to feed on each rat on two successive days. Most of the insects were of the species *Ceratophyllus fasciatus*, but some *Xenopsylla cheopis* were included.

Thirteen experiments were made; nine of the rats died of plague.

The proportion of infections is thus seen to rise with the number of opportunities (here 40) for infected fleas to feed upon the animal.

In the course of our experiments we made the observation that, whereas certain of our fleas sucked energetically and persistently, no blood entered their stomachs, but the oesophagus became unusually distinct. Usually, during feeding, the latter can only just be seen with a hand-lens as a fine red streak in the younger and more transparent fleas. The insects showing abnormality in this respect were, on removal to their tube, specially marked. On dissecting them a curious condition was discovered. Their proventriculi were blocked with what proved to be a solid culture of plague, and the oesophagi were more or less distended with fresh-clotted blood. (See Text-figs. 3 and 4, page 436, and Plates XXV and XXVI.)

It occurred to us that fleas whose proventriculi were obstructed with plague-culture were likely to be responsible for the conveyance of infection, so we next turned our attention to those insects which presented this interesting pathological condition. At the same time we made a study of the condition and how it is brought about, but this will best be dealt with at a later stage of our paper.

*Experiments with fleas suffering from obstruction in the proventriculus.*

Two methods of diagnosing the existence of obstruction were open to us. (1) By allowing a number of fleas to feed under supervision upon a rat, selecting those which could not satisfy their thirst and therefore when disturbed during the act of sucking would immediately start again at a fresh situation. (2) By examining under the microscope the fleas lying on their sides in a drop of water. Under these circumstances the obstruction could be actually seen owing to the dark brown colour of the alkaline haematin adsorbed due to the growth of plague bacilli. (See Figs. 2, 3 and 4, page 436.)

Having selected "obstructed" fleas from our supply of infected insects (they were already identified by a number) one or two were fed upon each of a series of rats, on one, or two, or sometimes three days in succession. Series IV gives the details of the experiments with fleas of the species *Xenopsylla cheopis*; and Series V those with the species *Ceratophyllus fasciatus*. As may be seen from the protocols below, every rat became infected with the former and one in six with the latter.

Although we have gained the impression throughout our experiments that infection is more easily produced by *Xenopsylla cheopis*, these figures alone are too small to warrant this conclusion. Under the conditions of our experiments obstruction of the proventriculus by plague culture certainly occurred more readily in the case of fleas of the species *Xenopsylla cheopis* than with those of *Ceratophyllus fasciatus*. The former is also a more persistent feeder if starved. While individuals of the species *Xenopsylla cheopis* would frequently renew their attempts six, eight or more times if disturbed, those of the latter species usually became restless and tried to get away after three or four attempts. In one or two cases, however, individual fleas of the species *Ceratophyllus fasciatus* were almost as persistent as those of *Xenopsylla cheopis*.



SERIES IV.

*Experiments with specimens of Xenopsylla cheopis having a blocked proventriculus.*

The figures 69 etc. in the tables below refer to the identification number of the flea. Where more than one number occurs in the same column it signifies that two fleas were fed upon the animal.

No. of rat	Days on which fleas fed and rats died						
	1	2	3	4	5	6	7
(1)	69	—	68 and 70	—	Died of pest	—	—
(2)	67	67	103 and 93	—	—	—	Died of pest
(3)	67	67	103 and 93	—	—	—	Died of pest
(4)	103 and 93	—	—	—	—	Died of pest	—

SERIES V.

*Experiments with specimens of Ceratophyllus fasciatus having a blocked proventriculus.*

No. of rat	Days on which fleas fed and rats died						
	1	2	3	4	5	6	7
(5)	64	—	—	—	—	—	lived
(6)	64	—	—	Died of pest	—	—	—
(7)	64	—	—	—	—	—	lived
(8)	94	—	94	—	—	—	lived
(9)	94	—	94	—	—	—	lived
(10)	94	—	94	—	—	—	lived

We finally varied the experimental conditions and allowed one or two "obstructed" fleas to attempt to feed once upon a succession of rats on the same day, the rats being thereafter segregated for observation.

SERIES VI. Two specimens of *Xenopsylla cheopis*, Nos. 95 and 103, were given two minutes each upon the shaved abdomen of eight rats in succession. Three contracted plague.

SERIES VII. On the same day as Series VI *Xenopsylla cheopis*, No. 95, was given the same opportunities on a further four rats. None contracted plague.

SERIES VIII. Two of three *Xenopsylla cheopis*, Nos. 105, 109 and 120, were allowed to feed upon nine rats in succession. Three died of plague.

SERIES IX. Two of three *Ceratophyllus fasciatus*, Nos. 99, 111 and 113, were given the opportunity to feed upon nine rats in succession. Three contracted plague.

The above experiments show that, given a flea in this pathological condition, the probability that it will convey infection is high. In considering the risk it must be borne in mind that in experiments VI to IX the fleas only had the chance of making one puncture and a time limit was imposed. Loose in the fur of an animal the insect would make a number of punctures in its efforts to satisfy its thirst.

*The development of the plague bacillus in the alimentary canal of the flea and the pathological condition thereby brought about.*

The large number of fleas which we dissected in order to ascertain the presence of infection, furnished us with considerable material to study this subject, and the interesting pathological condition which came to light stimulated us to prosecute this enquiry with diligence.

The mouth-parts and alimentary canal of the insect have been described in detail, and figured in a paper by the Commission which appeared in this *Journal* in 1906. For the appreciation of the curious pathological condition we have discovered a short description of the organs of alimentation will suffice. The diagram, Text-fig. 1 below, shows the general arrangement of the digestive system of the insect.

The mouth is situated at the attachment of the appendages, the apposition of which forms the piercing organ and sucking tube. From the mouth the pharynx passes upwards to the pump, the muscles actuating which are attached to the exo-skeleton along the curvature of the head. By the coordinated contraction of these muscles from before backwards, and the elastic recoil of the chitinous walls of the pump, the blood is sucked up the tube formed by the piercing organs and propelled backwards along the narrow oesophagus through the proventriculus into the stomach. The proventriculus is provided internally with a series of hair-like cells, broad at the base, fine at the free extremity and covered with chitin. These are arranged radially in seven rows, one above the other (see Plate XXVI, figs. 1 and 2), and curve posteriorly, their points touching and projecting into the stomach. The encircling bands of muscle contract the proventriculus until the tooth-like cells meet, and these circles of long-curved epithelial cells form an efficient valve between the proventriculus and stomach. Normally this valve is competent, and the energetic peristaltic contractions of the stomach, which take place during digestion, do not drive any blood back into the oesophagus. External to these tooth-like cells is a basement membrane, and outside this a series of bands of large striated muscle cells arranged circularly.

When watching the act of sucking, the proventriculus appears as a pulsating red globe, but it is not easy to determine whether the muscles of this organ actively participate or whether the appearance is merely due to the intermittent expansion of the organ as the blood is propelled into it by the pharyngeal pump.

The stomach is nearly as long as the abdomen, and its capacity depends upon its state of distension. It is lined internally with a single layer of epithelial cells of irregular shape and full of granules. In the distended stomach they are much flattened out. Outside this are two layers of muscles, the internal circularly and the external longitudinally arranged. Where the stomach joins the intestine four long malpighian tubes arise. Posterior to the stomach is a thin walled intestine of about the same length as the former, terminating in a much wider rectum with its six rectal glands.

Naturally we have not been able to follow the development of the bacilli in the alimentary canal of any one flea fed on septicæmic blood, and the following description is built upon the numerous observations we have made of insects in different stages of the infection. On examining the contents of the stomach of a flea a day or two after it has fed upon infected blood, clusters of minute brown specks darker in colour and firmer in consistency than the rest of the contents are visible with a magnification of 16 diameters (see the Text-fig. 1, p. 436). These, on examination with an immersion lens after staining, are seen to consist of plague bacilli (see Plate XXIV, figs. 3 and 4).

Later the stomach and proventriculus show definite jelly-like masses of a brown colour. These masses are possessed of considerable cohesion, and are with difficulty teased out so as to make a film suitable for microscopical examination. Plate XXIV, fig. 4, is a typical representation of the edge of such a mass, which is seen to be a piece of solid bacterial culture. The growth of such a culture-mass increases and, owing to its brown colour from adsorbed hæmatin, is very obvious on dissecting the stomach, and may often be quite readily seen when the entire flea is cleared and examined by transmitted light under a magnification of 20 diameters. The Text-figs. 1 to 4 p. 436, have been drawn from such preparations and represent stages in the development of the condition. The plague-culture grows in the proventriculus as well as in the stomach, and in Fig. 3 it is shown filling the whole of the stomach and proventriculus. Owing to its gelatinous consistency it not infrequently leads to incompetence and even complete blocking of the proventricular valve, as shown in figs. 1—6 Plate XXV, and fig. 2 Plate XXVI.

What precisely happens is seen on referring to Plate XXVI, figs. 1 and 2, which represent camera lucida drawings under a higher magnification of transverse and longitudinal sections of proventriculi in this pathological condition. Fig. 1 is from an early stage of infection. Between the tooth-like epithelial cells numerous bacilli may be seen. Later their

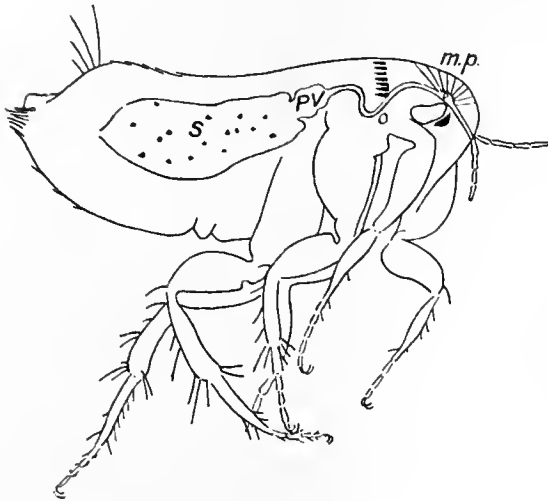


Fig. 1.

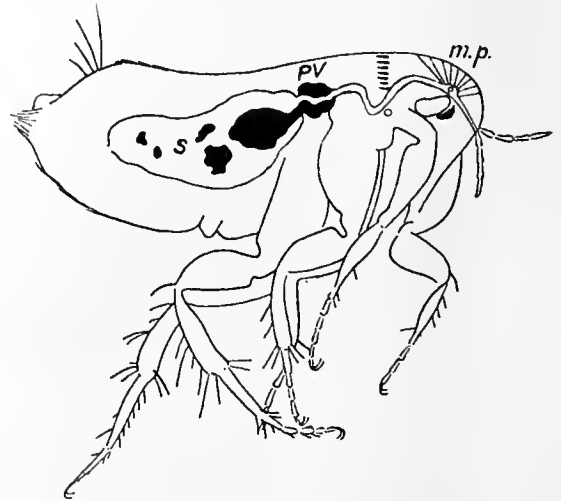


Fig. 2.

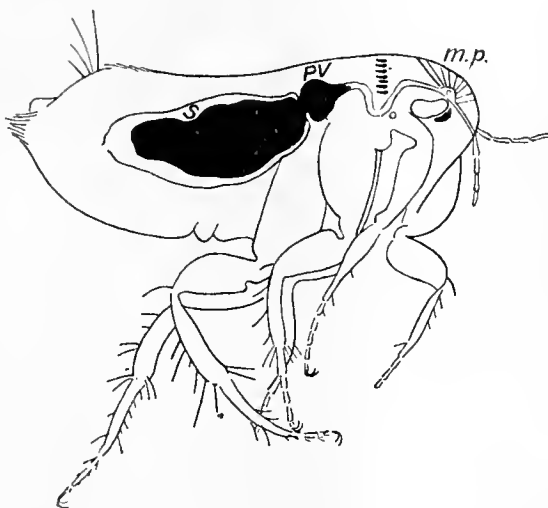


Fig. 3.

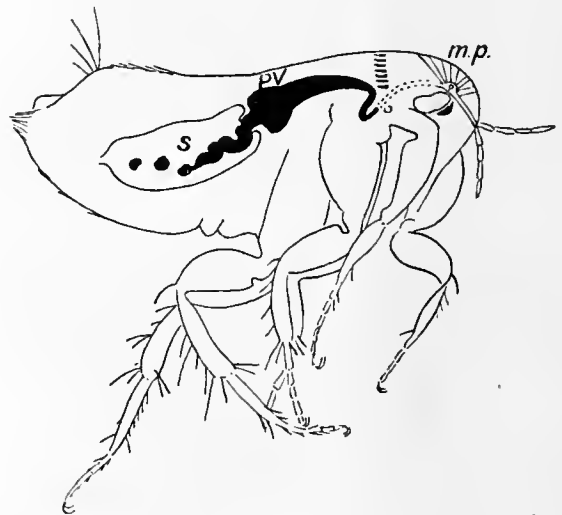


Fig. 4.

multiplication occurs to such an extent that these cells are widely separated and the proventriculus distended with culture. At the same time its lumen is obliterated, as is well seen in fig. 2 which represents a longitudinal section of an advanced condition. In this case the plug of culture extends into the oesophagus, and is capped by a clot of fresh blood which the insect had taken in just prior to the time the preparation was made.

Although, with the proventriculus obstructed in this manner, fresh blood cannot find its way into the stomach, this does not prevent the insect sucking, as the pump which aspirates blood up the sucking-tube and propels it into the stomach is situated in the pharynx. On the contrary, the flea suffers from thirst and is persistent in its efforts to satisfy this appetite, but only succeeds in distending the oesophagus. The blood in the distended oesophagus may flow out again on cessation of the sucking act, and we have seen drops of blood escape from the mouth-parts of "blocked" fleas when the insect withdrew its proboscis. Generally, however, sufficient time has elapsed for clotting to occur, and some blood remains in the oesophagus. Figs. 1 to 6 Plate XXV exhibit in diagrammatic form types of the appearances presented on dissecting out the alimentary canal. The plague-culture is sepia-brown in colour and easily distinguishable. The fresh blood which has recently been taken in is red.

The significance of these observations is obvious in view of our experiments, showing that fleas which are in this abnormal condition are particularly liable to transmit plague. In such fleas the oesophagus is infected with plague and fresh blood introduced becomes contaminated. Given the opportunity, the insects suck blood again and again, and if the pharyngeal pump ceases for a moment, some of the blood will by the elastic recoil of the oesophageal wall be driven back into the wound and carry with it plague bacilli.

The obstruction to the alimentary canal does not necessarily occasion the death of the insect, and, if kept at a cool temperature and in a moist atmosphere, the insects live for many days in this condition. We kept our specially selected fleas in a cool room at 10° C. In course of time the culture of plague obstructing the proventriculus undergoes autolysis and the passage is re-established. The obstructive process may, however, recur.

We have made experiments in which infected fleas were kept at different temperatures to learn whether they ever became free from bacilli. These fleas lived as long as 50 days at from 10° C. to 15° C. and 23 days at 27° C., and died infected.

Our results may be briefly summarised as follows:

Under conditions precluding the possibility of infection by dejecta it was found that two species of rat-fleas, *Xenopsylla cheopis* and

*Ceratophyllus fasciatus*, fed upon septicaemic blood, can transmit plague during the act of sucking, and that certain individuals suffering from a temporary obstruction at the entrance to the stomach were responsible for most of the infections obtained, and probably for all.

In a proportion of infected fleas the development of the bacilli was found to take place to such an extent as to occlude the alimentary canal at the entrance to the stomach. The culture of pest appears to start in the intercellular recesses of the proventriculus, and grows so abundantly as to choke this organ and extend into the oesophagus. Fleas in this condition are not prevented from sucking blood as the pump is in the pharynx, but they only succeed in distending an already contaminated oesophagus, and, on the cessation of the pumping act, some of the blood is forced back into the wound. Such fleas are persistent in their endeavours to feed, and this renders them particularly dangerous. Fleas suffering from obstruction do not necessarily perish, and in course of some days the culture obliterating the lumen of the proventriculus may autolyse and the passage again become pervious. They are, however, incapable for the time being of imbibing fresh fluid, and are, therefore, in danger of drying up if the temperature is high and the degree of saturation of the atmosphere low. Although, as far as our observations go, they withstand desiccation quite as well as normal fleas which are not fed, their length of life must be short directly hot, dry weather sets in, and we are led to wonder whether this fact may not, to some extent, explain why in India epidemic plague is confined to the cooler and moister seasons, and particularly why in Northern and Central India the epidemics are abruptly terminated on the onset of the hot dry weather.

In conclusion we desire to express our indebtedness to our assistant, Mr D. J. Russell, who helped us in many of the experiments, and to Miss M. Rhodes, who made the drawings and diagrams which illustrate this paper.

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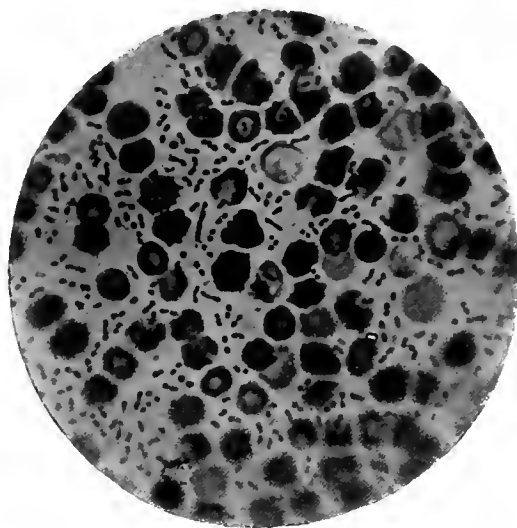


Fig. 1.

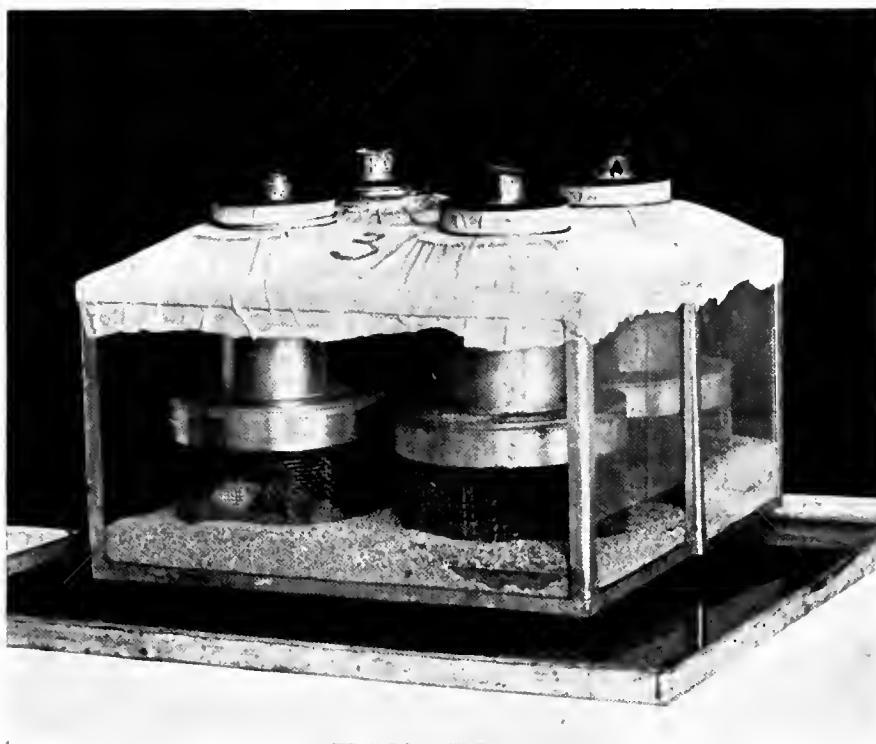


Fig. 2.

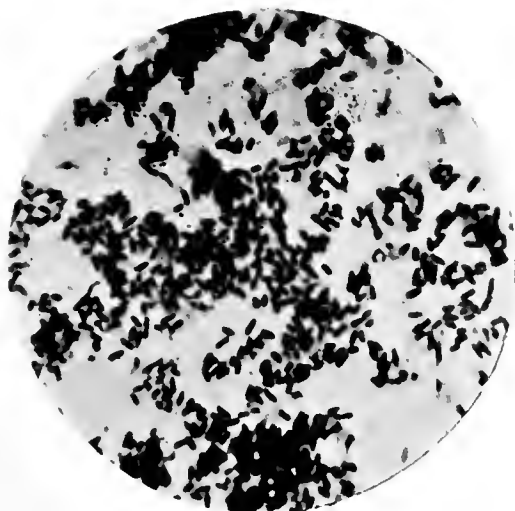


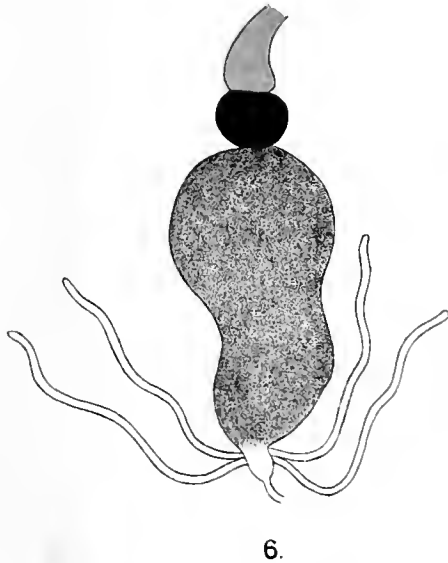
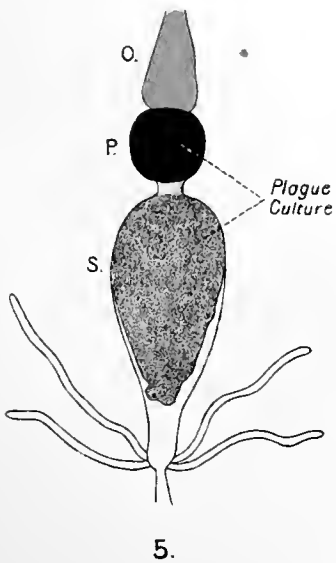
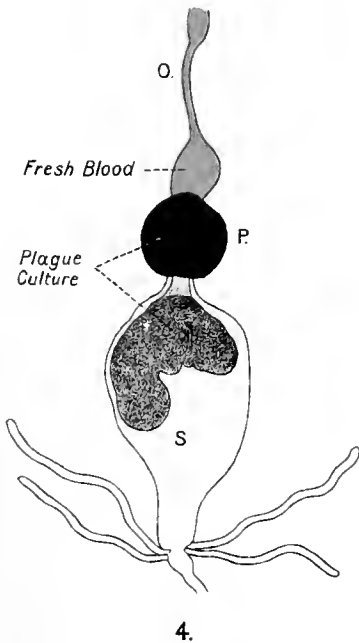
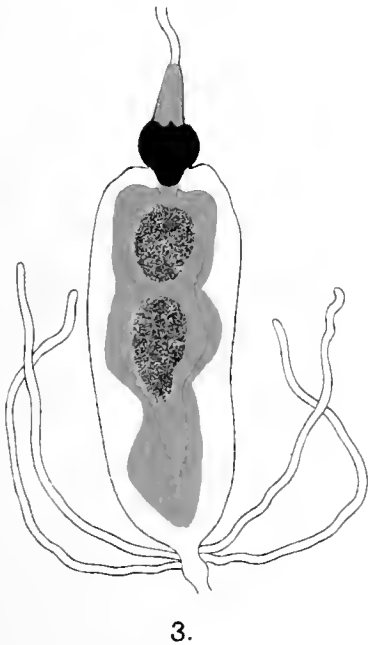
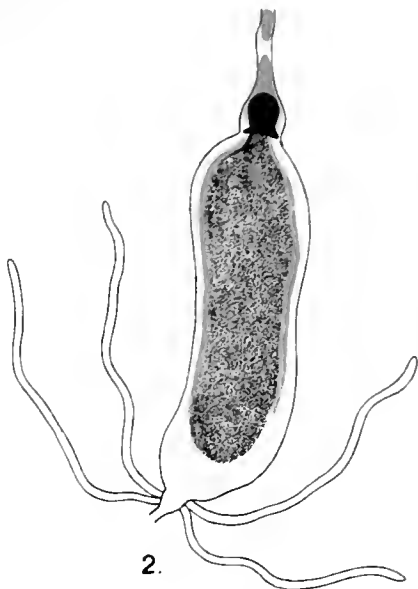
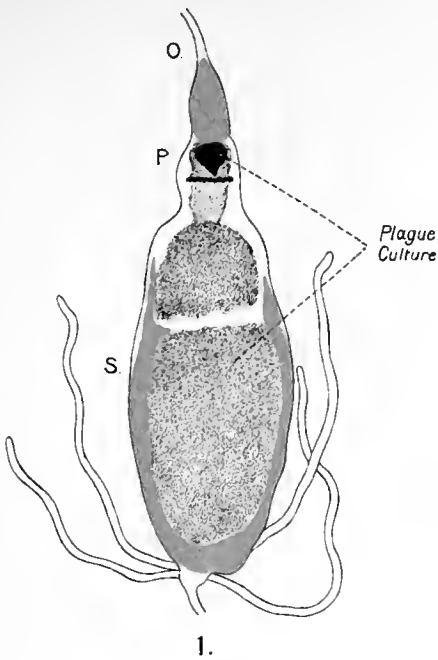
Fig. 3.



Fig. 4.









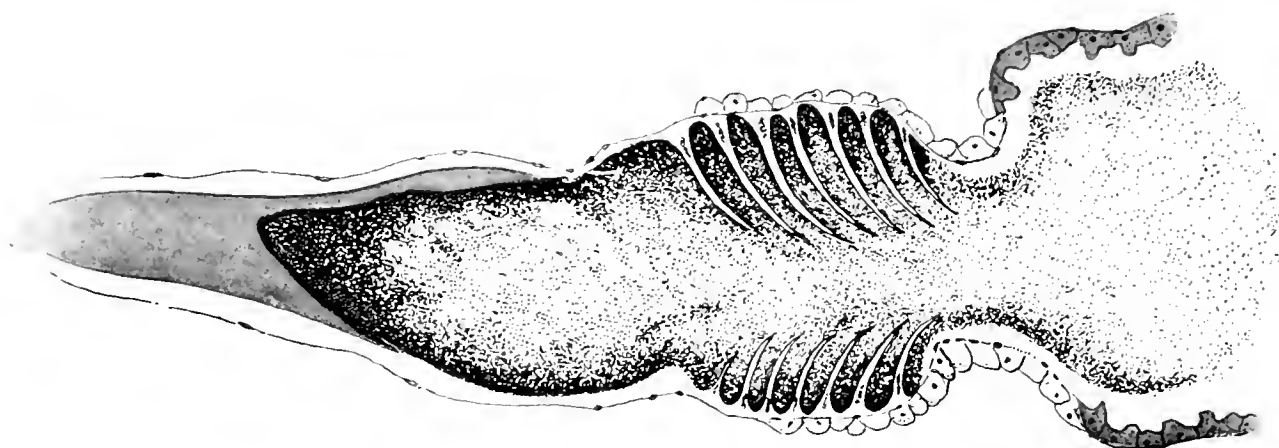


Fig. 2.

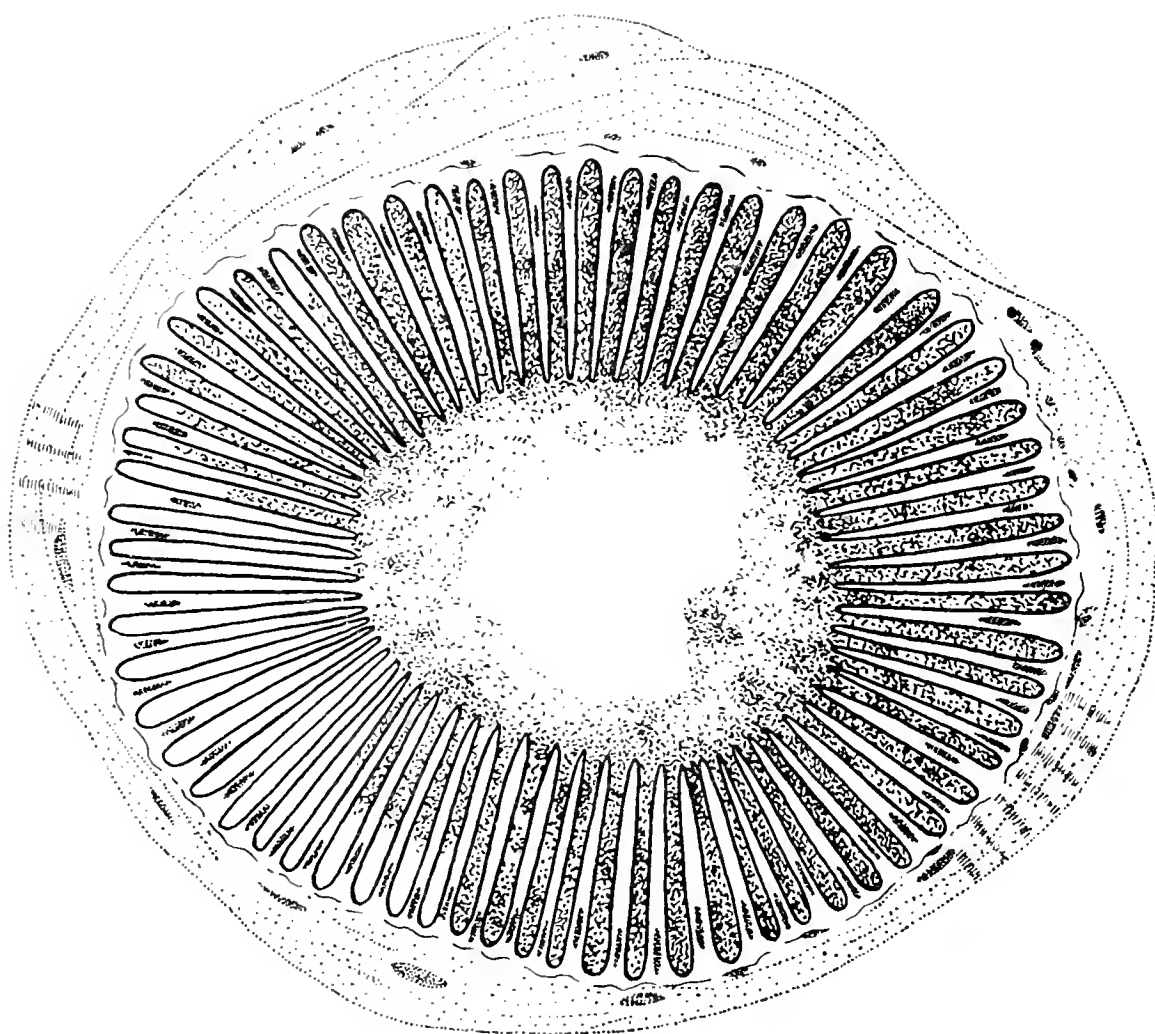


Fig. 1.

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## DESCRIPTION OF PLATES XXIV—XXVI.

- Plate XXIV. Fig. 1. Type of plague septicaemia in the mice upon which the fleas were fed.  $\times 500$ .
- Fig. 2. Cages supported over a trough of lysol in which the fleas were bred and became infected.
- Figs. 3 and 4. Smears of contents of the stomachs of infected fleas showing plague bacilli.  $\times 1000$ .
- Plate XXV. Figs. 1—6. Types of the appearances seen on dissecting out the stomachs of "obstructed fleas." Plague culture shown dark grey to black. Fresh blood distending the oesophagus red.
- Plate XXVI. Fig. 1. Transverse section of proventriculus of *C. fasciatus* in early stage of infection; showing striated muscle fibres circularly disposed; tooth-like epithelial cells covered with chitin and plague bacilli growing between cells commencing to block lumen.
- Fig. 2. Longitudinal section of oesophagus, proventriculus and portion of stomach of *C. fasciatus* in late stage of infection. Fresh blood recently imbibed by the insect is seen on top of a cap of plague culture which projects into oesophagus.

(For detailed description see text p. 434.)

LXVIII. INFLUENCE OF THE MEDIUM IN WHICH  
*B. PESTIS* IS PROPAGATED UPON ITS VIRULENCE.

By SYDNEY ROWLAND, M.A., M.R.C.S.,  
*of the Lister Institute.*

IN a previous paper (this *Journal*, Plague Supplement, No. III, p. 403) I pointed out that plague bacilli grown on ordinary laboratory media although affording a satisfactory antigen wherewith to vaccinate against virulent bacilli grown on similar media, produce an antigen conferring but slight immunity upon rats against a strain of organisms propagated in the body or in body fluids. This suggests a serious defect in ordinary vaccines, for the organisms employed in their preparation are usually those propagated in broth or on broth agar.

In the same paper it was further shown that the antigen prepared from bacilli grown in serum-containing media is more nearly homologous to that produced by organisms growing in the body, for it is capable of conferring a considerable degree of immunity against such organisms. Thus, a dose of living organisms (5 millions) recovered direct from the spleen of a rat dead of plague killed 19 out of 20 normal rats. The same organisms in similar dose only killed, however, 50 % of rats protected by one injection of an antigen obtained from bacilli grown in serum-containing media and only 12 % of another series of rats that had received two successive doses of the same antigen.

These results have led me, starting from an ordinary laboratory strain, to attempt the cultivation outside the body of a race of organisms of full virulence comparable with that exhibited by organisms as they occur in the spleen of a rat dead of pest. My endeavour was also to discover the conditions necessary for the maintenance of the virulence of such a culture. Until this can be done, the preparation of a vaccine whether composed of the whole bacilli or by the extraction of the corresponding antigen cannot be advantageously proceeded with.

I give here a short account of some experiments that have been made with this object, although from the main point of view of the research the enquiry is unfinished.

*The virulence of the stock agar culture employed.* The parent culture from which all the subcultures were made was kept on nutrient agar. It was transplanted every week and maintained in the dark at the temperature of the laboratory. Derived originally from a case of plague in the Mahratta Hospital, Bombay, it has been propagated in this way ever since its isolation three years ago. With propagation on ordinary laboratory media the virulence of plague cultures depreciates, at first rapidly, and subsequently more gradually. The experiments detailed below were not commenced until the culture had been growing on agar for more than two years and had acquired the low level of virulence characteristic of laboratory strains. During the time that these experiments lasted the standard dose of organisms from the agar growth killed 55 % of rats when suspended in salt solution and injected subcutaneously.

The average time of death was six days and the character of the infection sub-acute, the animals dying without extensive invasion of the organs by plague bacilli. This culture formed the starting point of all the experiments and may be referred to as "Agar stock."

*Influence of propagation in various media upon the virulence of the "Agar stock" strain of B. pestis.* When the culture "Agar stock" was propagated in broth the injection of one million organisms killed 70 % of rats in an average time of 5 days. It has continued to do this for 3 years. Broth cultures are consistently more virulent than those grown on agar.

If a small proportion of serum proteins be added to the broth in which the bacilli are grown an immediate rise in virulence results. Thus, the addition of 10 % normal horse serum, previously heated to 55° C., for half an hour has consistently [in five series of experiments] produced a strain, of which one million bacilli killed 9 out of 10 rats in an average time of 4.5 days. This indicates a rise in virulence far greater than that indicated by the numerical difference obtained, for in any given number of rats there is a proportion of resistant or relatively immune individuals which are difficult to infect.

TABLE I. *Influence of subsequent propagation in various media upon the virulence of B. pestis "Agar stock."*

Dose = approximately 1,000,000 bacilli.

Expt. No.	Medium in which the bacilli used for the test were propagated	No. of rats inoculated	No. of rats dying	% rats dying	Time elapsing before death—in days
1	Nutrient broth	100	70	70	5
2	Nutrient broth containing 10 % horse serum	50	45	90	4·5
3	Fraenkel's medium + 0·5 % pure crystalline egg-albumen	20	18	90	3·7
4	Salt solution containing 10 % egg-white	10	8	80	4·6
5	Horse serum after filtration through gelatine filter	10	9	90	3·7
6	Ox-spleen juice	30	30	100	3

This result which has been confirmed repeatedly suggested that a pure protein medium might also yield a virulent strain. Trial was accordingly made of the virulence of a growth in Fraenkel's medium<sup>1</sup> to which 0·5 % crystalline serum albumen had been added. One million organisms from this culture killed 18 out of 20 normal rats in an average time of 3·7 days, whilst the same dose of a culture in 10 % egg-white in salt solution killed 8 out of 10 in an average time of 4·6 days. Both of these cultures are distinctly more virulent than the agar stock from which they were inoculated (see Exps. 3 and 4, Table I).

In further experiments which, with the foregoing, are summarised in Table I a medium was prepared which contained the normal salts of serum with a minute trace of the natural serum proteins, viz., the liquid obtained by passing horse serum through a gelatine filter. A dose of one million bacilli from the culture in this medium killed 9 out of 10 rats in an average time of 3·7 days (Exp. 5).

A consideration of these results suggested that for the attainment of its full virulence the plague bacillus requires the presence of some protein in its food, and in this connection it should be remembered that the plague bacillus contains a proteolytic enzyme of considerable activity.

<sup>1</sup> *B. pestis* will not grow in Fraenkel's medium alone.



It was natural to suppose that if a laboratory strain of plague could be educated to grow in perfectly fresh serum, organisms of still further enhanced virulence would be produced. Such was, however, not found to be the case.

*Influence of cultivation in fresh rat serum upon the virulence of B. pestis of varying origin.*

(a) "Agar stock." When the culture "Agar stock" was inoculated into fresh normal rat-serum, growth had usually occurred by the next day but the virulence of the organisms was generally found to be much diminished. The usual dose of one million bacilli was employed (see Exp. 1, Table II).

TABLE II. *The effect of cultivation in fresh rat-serum on the virulence of the plague bacillus of various origin.*

Expt. No.		No. of rats in- oculated	No. of rats dying	% rats dying	Average time elapsing before death—in days
1	"Agar stock"	20	11	55	6
	Do. grown in fresh rat-serum	90	22	24	6
2	Broth culture of "Agar stock"	10	9	90	4
	Do. grown in fresh rat-serum				
	1 day incubation	10	5	50	6.6
	2 days' ,,	10	2	20	6
3	Serum culture of "Agar stock"	10	6	60	6.3
	Do. grown in fresh rat-serum				
	1 day incubation	Growth insufficient for experiment			
	2 days' ,,	10	1	10	5
4	Organisms from spleen of rat dead of plague	10	9	90	2.9
	Do. grown in fresh rat-serum	10	9	90	3
5	Heated horse serum culture of "Agar stock"	20	20	100	3.5
	Do. grown in old unheated serum and subse- quently grown in fresh rat-serum	8	8	100	5
6	Spleen serum medium culture of "Agar stock"	30	30	100	2.7
	Do. grown in fresh rat-serum	10	10	100	4.4

NOTE. Experiments 2 and 3 were done at the same time on the same batch of rats and are strictly comparable one with another. Taking individual batches of experiments it sometimes is found that the serum culture of "Agar stock" varies in virulence. In Exp. 3 it was as high as 60%. This is exceptional but in this case fortunate as it brings out the depressing effect of subsequent cultivation in fresh serum more strikingly.

Starting from "Agar stock" a parallel series of cultures was made (a) in broth (Exp. 2, Table II) and (b) in fresh rat-serum (Exp. 3

Table II). After 24 hours' incubation at 36° C. the broth culture contained innumerable organisms, while the serum culture contained about 16 million organisms per cubic centimetre. The broth culture in a dose of one million organisms killed 9 out of 10 rats while the serum culture in the same dose killed only 6 out of 10. The same quantity (one million bacilli) of both cultures was then inoculated into fresh rat-serum. After 24 hours' incubation the serum that was inoculated with the broth culture contained more than 200 million organisms per c.c., and the virulence was such that one million organisms killed 5 out of 10 rats. The serum that had been inoculated with the serum culture, on the other hand, contained so few organisms that none could be found in the counting chamber. Both cultures were replaced in the incubator.

After a further period of 24 hours' incubation the serum culture derived from the broth culture contained so many organisms that counting in the undiluted state was impossible, while the serum culture derived from the serum culture contained about 120 million organisms per c.c. The virulence of the former was such that one million organisms killed 2 out of 10 rats whilst that of the latter such that the same number of organisms killed but 1 out of the same number. In this experiment the depressing effect of culture in fresh normal serum is obvious.

(b) *Body strain.* The plague bacillus as it exists in the body of a rat dead of plague is resistant to this depressing action of normal serum outside as the following experiments show.

The spleen of a rat dead of plague was emulsified in salt solution and the organisms obtained in suspension by partial centrifuging. One million of these organisms killed 9 out of 10 rats in an average time of 2.9 days. The bacilli were then propagated in fresh normal rat-serum, in which they grew luxuriantly and killed 9 out of 10 rats in an average time of 3 days (Exp. 4, Table II).

If these results be compared with the results of propagating broth-grown organisms in fresh rat-serum they present a striking contrast.

(c) *Other virulent strains, artificially produced.* Some success in producing a strain of organisms of high virulence and capable of resisting the depressing action of fresh rat-serum was obtained by interposing between the parent culture "Agar stock" and the final culture in fresh rat-serum, cultures on various media that might be supposed to educate the organisms to grow in fresh serum.

The following experiment (Exp. 5, Table II) may be quoted as

typical of many attempts in this direction. The culture "Agar stock" was inoculated into normal serum that had been previously heated to 55° C. for half an hour. The usual dose of the culture thus obtained killed 20 out of 20 normal rats, and after inoculation into rat-serum that had been kept a month, yielded a luxuriant growth. This was then inoculated into fresh rat-serum and good growth took place in 24 hours. This latter culture killed 8 out of 8 normal rats in an average time of 5 days, a degree of virulence approximating to that displayed by the organisms direct from the body of an infected animal. The killing time is longer but the virulence of the culture had successfully withstood the depressing action of the fresh rat-serum. Several other experiments have yielded a similar result.

The most promising results have been obtained by sowing direct from "Agar stock" into a medium prepared by extracting the minced spleen of a rat with the fresh serum of the rat that afforded the spleen. In one case a dose of one million bacilli of a strain propagated in such a medium killed 10 out of 10 rats in an average time of 2·7 days. In another experiment the same dose of a similar culture killed 20 out of 20 rats in an average time of 2·9 days. Apparently the depressant effect of fresh serum on virulence is counteracted by the addition of spleen juice.

When these virulent cultures in spleen-containing media were transferred to fresh rat-serum (without the spleen) they maintained their virulence for 24 hours in marked contrast to the influence of the same medium upon broth-grown organisms. Thus a culture in spleen serum which killed 10 out of 10 normal rats in an average time of 3 days, when subcultured in fresh rat-serum still killed 10 out of 10 normal rats, the average time of death rose, however, from 3 to 4·4 days. The character of the infection was, however, acute and of the same order as that produced by the original spleen serum culture.

In place of the rat-spleen, use has also been made of the spleen of the ox which can be obtained in larger quantity. A medium was prepared by mincing a fresh spleen and pressing out the juice. The "Agar stock" strain, when grown in this medium, produced a race the usual dose of which killed 10 out of 10 normal rats in an average time of 3 days—the same degree of virulence as that obtained by growing in the rat-spleen medium. The growth in spleen media is, however, not always of this high degree of virulence. The cause of the irregularity has so far resisted all attempts to unravel it.

The foregoing experiments, incomplete though they are, lead to one important conclusion. *The serum of the normal rat has the power of depressing the virulence of the plague bacillus. This is particularly marked with organisms propagated in broth. Whereas broth organisms lose their virulence after 24 hours in fresh serum, bacilli direct from the body of an infected animal are not greatly affected in this time. The suggestion is therefore made that infection of a rat by plague follows or not according as the infecting organisms when pitted against this property of the rat's serum succeed or fail in breeding out a strain of organisms which can resist this depressing action. The balance between these two possibilities would appear to be a delicate one.*

LXIX. A STUDY OF THE BIONOMICS OF THE COMMON RAT FLEAS AND OTHER SPECIES ASSOCIATED WITH HUMAN HABITATIONS, WITH SPECIAL REFERENCE TO THE INFLUENCE OF TEMPERATURE AND HUMIDITY AT VARIOUS PERIODS OF THE LIFE HISTORY OF THE INSECT.

By A. BACOT, *Entomologist, Lister Institute of Preventive Medicine.*

(With Plates XXVII—XXXIV, 12 Charts and 3 Text-figures.)

TABLE OF CONTENTS.

- I. Introduction.
- II. Species and source of the fleas investigated.
- III. Apparatus and methods employed in making the observations.
  - (a) Range of temperature and humidity.
  - (b) General arrangement and management of breeding cages.
  - (c) Methods employed in rearing fleas and experimenting with them.
    1. Obtaining and hatching eggs.
    2. Food and treatment of larvae.
    3. Obtaining and treatment of cocoons.
    4. Control and treatment of adults.
- IV. General observations on the bionomics of fleas.
  1. Eggs.
  2. Larvae.
  3. Cocoons.
  4. Adults.
- V. Experimental study of the influence of external conditions on the various stages.
  1. Eggs.
  2. Larvae.
    - i. Ability of newly hatched larvae to survive without food.
    - ii. Influence of temperature, humidity and nature of food supply on rearing.
    - iii. "Lagging" before spinning cocoons.
  3. Cocoons.
    - i. Influence of temperature, humidity and nature of previous history on duration of cocoon stage.
    - ii. Influence of immersion in water upon vitality during cocoon stage (*Ct. canis*).
    - iii. Influence of external conditions at different periods of larval existence upon the duration of the cocoon stage in *C. fasciatus*.
    - iv. Significance of "Hard" and "Soft" cocoons of *C. fasciatus*.

## 4. Adults.

- i. Duration of life unfed.
- ii. „ „ when fed.
- iii. „ „ if fed upon an alien host.
- iv. Attempts to induce fleas to feed upon material other than the blood of a living animal.
- v. Possibility of continued breeding in the absence of any host.
- vi. Fertility of males and survival of spermatozoa in the spermathecae of the female (*P. irritans*).
- vii. Influence of food supply on number and fertility of eggs (*P. irritans*).

VI. Influence of low temperature on the various stages.

VII. Summary.

## SECTION I. INTRODUCTION.

THIS research was undertaken at the instance of the Advisory Committee for the investigation of plague in India, its object and scope being to ascertain the effects of varying conditions of temperature and humidity on the species of fleas associated with rats and man: to trace if possible the critical temperatures and humidities which allow of or prevent their breeding, and further to ascertain if possible in which of the several stages of the flea's life history, whether as ova, larvae, pupae or imagines, the effects of extreme conditions of drought and heat proved most fatal. It was also expected that evidence might be forthcoming as to a probable aestivating or hibernating stage, in which these species could tide over periods when heat, drought or cold prevent active breeding and cause the flea population to fall to a minimum, or to disappear entirely, as in the case of *Ceratophyllus fasciatus* in the Punjab, between the months of May and November.

The methods and apparatus employed together with general remarks with regard to rearing, habits and life history of the flea, are treated as a separate chapter at the close of the introductory remarks. The experiments on breeding were of two kinds, firstly a series of rearing experiments from the egg onwards under different conditions, and secondly definite tests carried out separately on the several stages. The former as well as the latter have, however, been arranged under the various headings of eggs, larvae, cocoons and adults for the sake of convenience in tabulation. The numbers employed in each experiment in addition to the date should render the continuation of the individual experiments in the subsequent stages easy to follow.

The species used for these experiments were chiefly *Pulex irritans*, *Ceratophyllus fasciatus*, and *Xenopsylla cheopis*. Some work was also carried out with *Ceratophyllus gallinae* and *Ctenocephalus canis*, as well as with *Ctenocephalus felis* on one occasion, but difficulties in feeding these species on their proper hosts in captivity have prevented a plentiful supply of eggs being obtained, and restricted the scope of the experimental work. One set of experiments was also performed with *Leptopsylla musculi* in the summer of 1912, in order to study the conditions determining emergence from cocoons and the length of the resting period.

## SECTION II. SPECIES AND ORIGIN OF THE FLEAS USED IN THE EXPERIMENTS.

*Ceratophyllus fasciatus* (Pl. XXVII, figs. 1 and 2). Some 30 adults were received from Prof. Minchin's stock (Lister Institute), which originated from fleas captured on rats trapped near Sutton Broad Laboratory, Norfolk, and a similar number were received from Prof. Nuttall's Laboratory at Cambridge.

*Pulex irritans* (Pl. XXVIII, figs. 1 and 2). A start was made with about a dozen specimens captured at Loughton and, although a few specimens were received from a friend in Hampshire, it is very doubtful if there is any infusion of blood from this source in the stocks used for the experiments, as the survivors of the specimens received were very feeble.

*Ctenocephalus canis* (Pl. XXIX, figs. 1 and 2), *Ctenocephalus felis* (Pl. XXIX, figs. 3 and 4) and *Ceratophyllus gallinae* are all of Loughton race.

*Leptopsylla musculi* (Pl. XXVII, figs. 3 and 4) were obtained from the Lister Institute.

*Xenopsylla cheopis* (Pl. XXVIII, figs. 3 and 4). Tubes containing living larvae of this species forwarded from India in the autumn of 1910 did not produce sufficient fleas to give an effective start, but specimens obtained subsequently by Dr Boycott from an English source have resulted in strong stocks, and the experiments have been performed with individuals of this race.

It is possible, even probable, that experiments on stocks from restricted localities may not give evidence of the full range of variation

of a species, which might enable it to adjust itself to the varying conditions of climate experienced in its geographical distribution. The known variation of habit in other insects, which meet needs called forth by slight differences within small areas, suggests this is likely to be the case<sup>1</sup>. On the other hand the wide range of variation in duration of the cocoon period, found present in the stocks of *C. fasciatus* and *P. irritans*, would afford ample opportunity for selective action when taken in conjunction with their powers of rapid multiplication. It would be simple in the face of a climate with an extreme range of conditions, such as is experienced in some parts of India, for a race of *C. fasciatus* to be produced in which rapidly emerging individuals were in the majority at one period of the year and those having a long delayed period of emergence constituted the bulk of the flea population at a succeeding period. It seems probable that this is the explanation of the recorded complete disappearance of *C. fasciatus* during the hot months in the Punjab (*Journal of Hygiene*, Vol. VIII. No. 2, May 1908, p. 241).

In the case of *C. fasciatus* breeding cages were started in July 1910, but it was not until the autumn of that year that a sufficient stock was available for the production of ova in the numbers required, while with *P. irritans* it was only with the commencement of spring 1911 that egg laying was on a satisfactory basis. In consequence, the early experiments which deal with very small numbers but cover as wide a range of conditions as possible are to be regarded in the light of an attempt to ascertain the direction in which future experiments, with larger numbers, might most profitably be carried out. They also afforded an opportunity of improving the methods of treatment and feeding. Many of the results are of considerable interest and, as in the case of resting cocoons, are valuable in themselves, in spite of the small numbers of fleas employed; but others are unsatisfactory, if not deceptive, for the coincidence of food tests with seasonal changes renders it uncertain as to whether the results are due to one cause or the other.

There was no evidence of a definite resting phase during the earlier experiments but rather of a general variability dominated by temperature. With larvae of *C. fasciatus* taken from the cages during the

<sup>1</sup> *E.g.* *Papilio machaon* is single brooded in Norfolk and double brooded in Cambridgeshire.



Winter 1910-11, there was, however, a distinct tendency to rest in the cocoon stage, and this was in great measure irrespective of the range of experimental conditions.

In one instance broods were ruined owing to a too drastic reduction in the humidity of an incubator; in another to the use of a sample of sand which proved inimical to the larvae; the cause of this was suspected to be the presence of sodium chloride.

It has been thought best to include all the experiments without exception, however clear the cause of failure might appear, in the hope that future workers in this new field of research may find the results of some value.

### SECTION III. APPARATUS AND EXPERIMENTAL METHODS EMPLOYED.

#### (a) *Range of external conditions; temperature and humidity.*

The experiments were carried out chiefly in four incubators, two of which were maintained at 75° F.<sup>1</sup>, but with differing degrees of humidity, and a similar pair at 85° F.

In addition use was made of a cellar, the Laboratory cupboard and one situated next to a chimney flue, the fire being unused between the months of May and September.

Since December 1910 use has also been made of an empty beehive in the garden.

INCUBATORS. For clearness the incubators have been designated by their temperature and their condition as regards humidity, thus: incubator 85 Wet; incubator 85 Dry; incubator 75 Wet; incubator 75 Dry. Records of temperature and humidity were taken twice daily, except on Sundays, when as a rule only one reading was made. These are set out in tabular form below, pp. 460-2, and graphically shown in Charts 1 and 2. A few records had to be discarded owing to the Wet bulb thermometers being out of order. The conditions aimed at were to keep one at 85° F. with humidity at .70; one at 75° F. humidity .75; one at 85° F. humidity at .55 to .60, and one at 75° F. with humidity at .50 to .55.

The degree of humidity in the air is according to Glaisher—"The ratio of the quantity of vapour present in any volume of the air to

<sup>1</sup> All temperature readings are on the Fahrenheit scale.

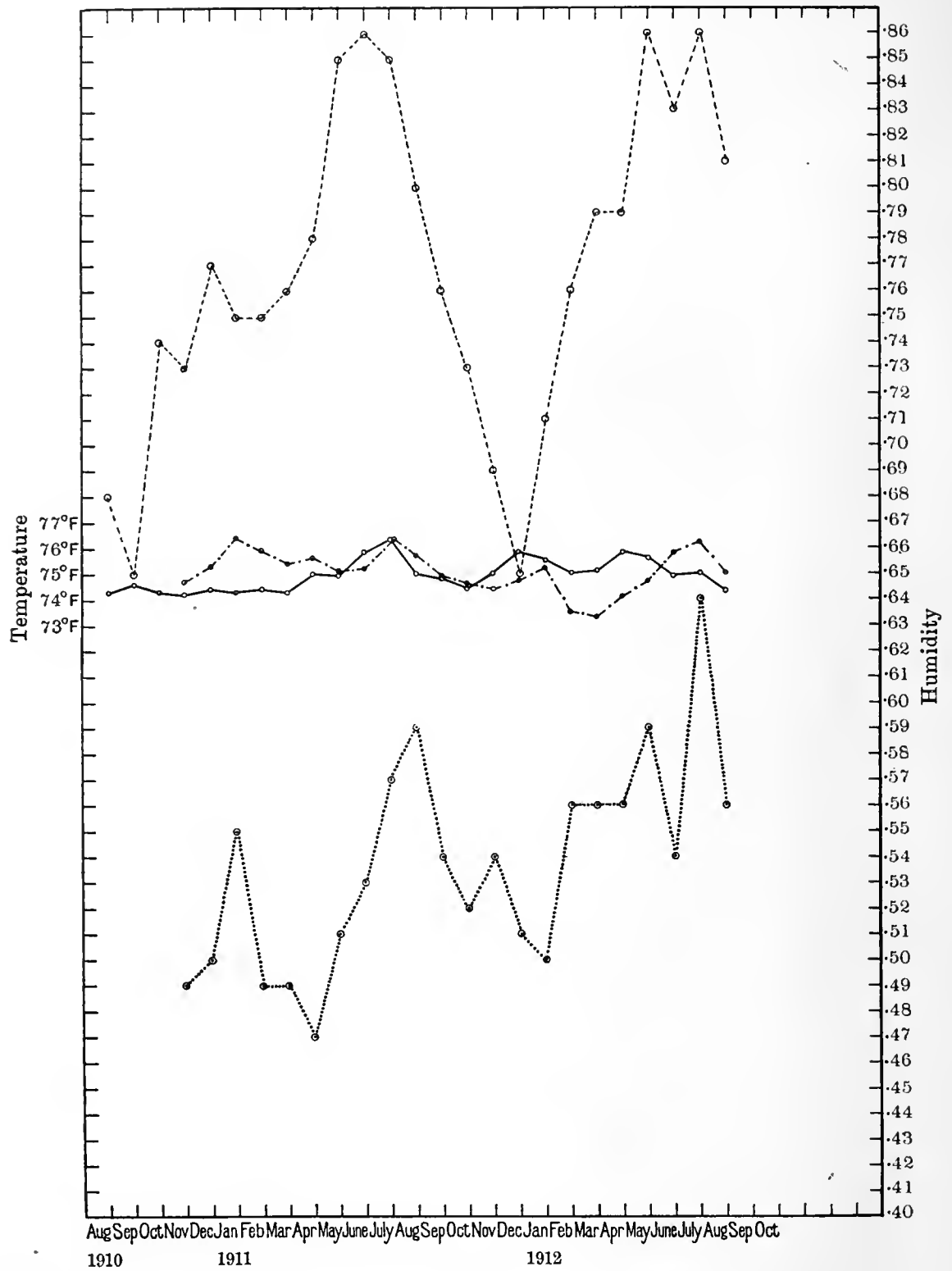


Chart 1.

Incubator 75 Wet.  
Incubator 75 Dry.

Temp. ————— Humidity - - - - -  
Temp. - - - - - Humidity . . . . .

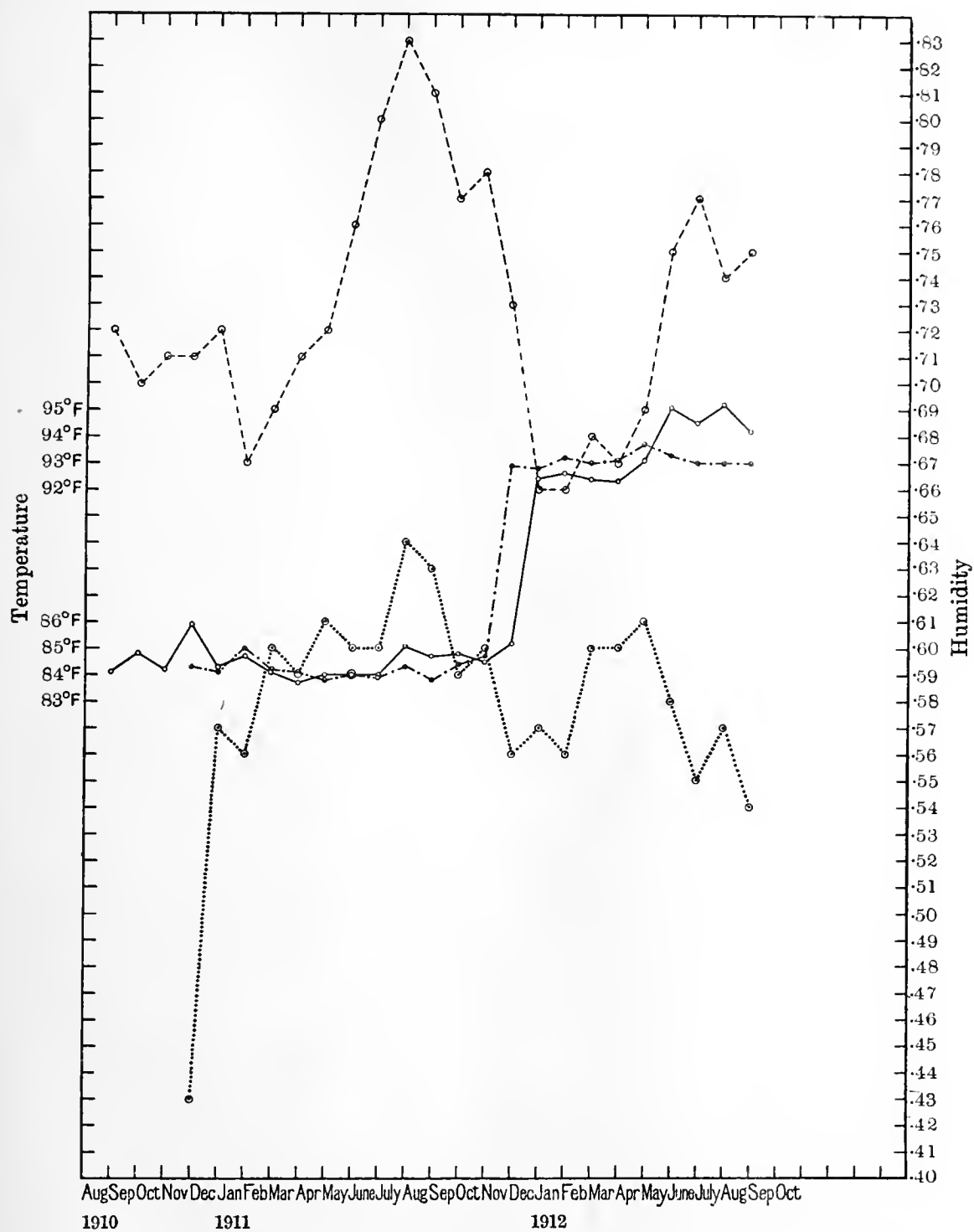


Chart 2.

Incubator 85/95 Wet.  
Incubator 85/95 Dry.

Temp. ————— Humidity - - - - -  
Temp. . . . . Humidity . . . . .

the quantity which would have been present in the same volume, had the air been completely saturated." The readings of the "Dry" and "Wet" bulb thermometers were taken and the percentage of humidity obtained from Glaisher's tables.

During November 1911 the temperature of the two hot incubators, 85 Wet and 85 Dry, was raised to 93° F. or thereabouts, as it was desired to make certain tests under conditions of greater heat; from the time of change onwards they are referred to as 93 Wet and 93 Dry—a few experiments that had been started at the lower temperature being finished at the higher.

Except during the hot summer and cold winter months, it will be seen that the averages are roughly in the neighbourhood of the desired figures. From the contrasted breeding experiment dealing with *P. irritans*, *X. cheopis* and *C. fasciatus* (Table XXV) it will be seen that the rise in humidity of 85 Dry and 75 Dry to .63 and .59 respectively in these two incubators was not sufficient to permit of any larvae surviving, so that the experiments were not really interfered with from this cause.

LABORATORY CUPBOARD. (Chart No. 3.) This is a rather cool and very draughty place with a cement floor, on a side of the building that never gets any direct sunshine on it. Its temperature tends to be rather lower than that of a room without a fire and its humidity during cool wet autumn or winter weather is well in excess of the incubators; it tends to be drier than the incubators during the spring and summer months. A glance at the experiments (Tables XVII and XXI) will show that, while the conditions are next to impossible for *C. fasciatus*, they are successfully surmounted by a percentage of *P. irritans*.

THE CELLAR. (Chart No. 4.) This gives an even range of temperature seldom differing by more than one or two degrees from night to day, and ranging from an average of 44° F. in February to 64° F. in August 1911 (four degrees higher than the summer temperature of the previous year). Its record of humidity is even more stable—being practically constant at .91 to .93. It has a bricked but uncemented floor and probably represents fairly well the conditions of cellarage prior to the free use of cement in modern buildings.

The conditions were found to be very favourable to all stages of the flea's life history with the exception of egg laying, and possibly hatching—save during the warm months—but development of the larvae is slow, so slow in some instances that it is a question whether the final failure of larvae to spin, as in experiment 6th October 1910 with

*P. irritans*, may not have been due to their food deteriorating during the long delay and their ultimate starvation.

WARM CUPBOARD. (Chart No. 5.) As will be seen from the tables, the range of conditions is not very wide, apart from the extremes of the summer of 1911. Normally the temperature is between 60° F. and 70° F. and its humidity not far from .55 to .60. Its conditions seem to be even less favourable for the hatching of eggs than the low humidity incubators 85 Dry and 75 Dry, and at least equally fatal to larval development.

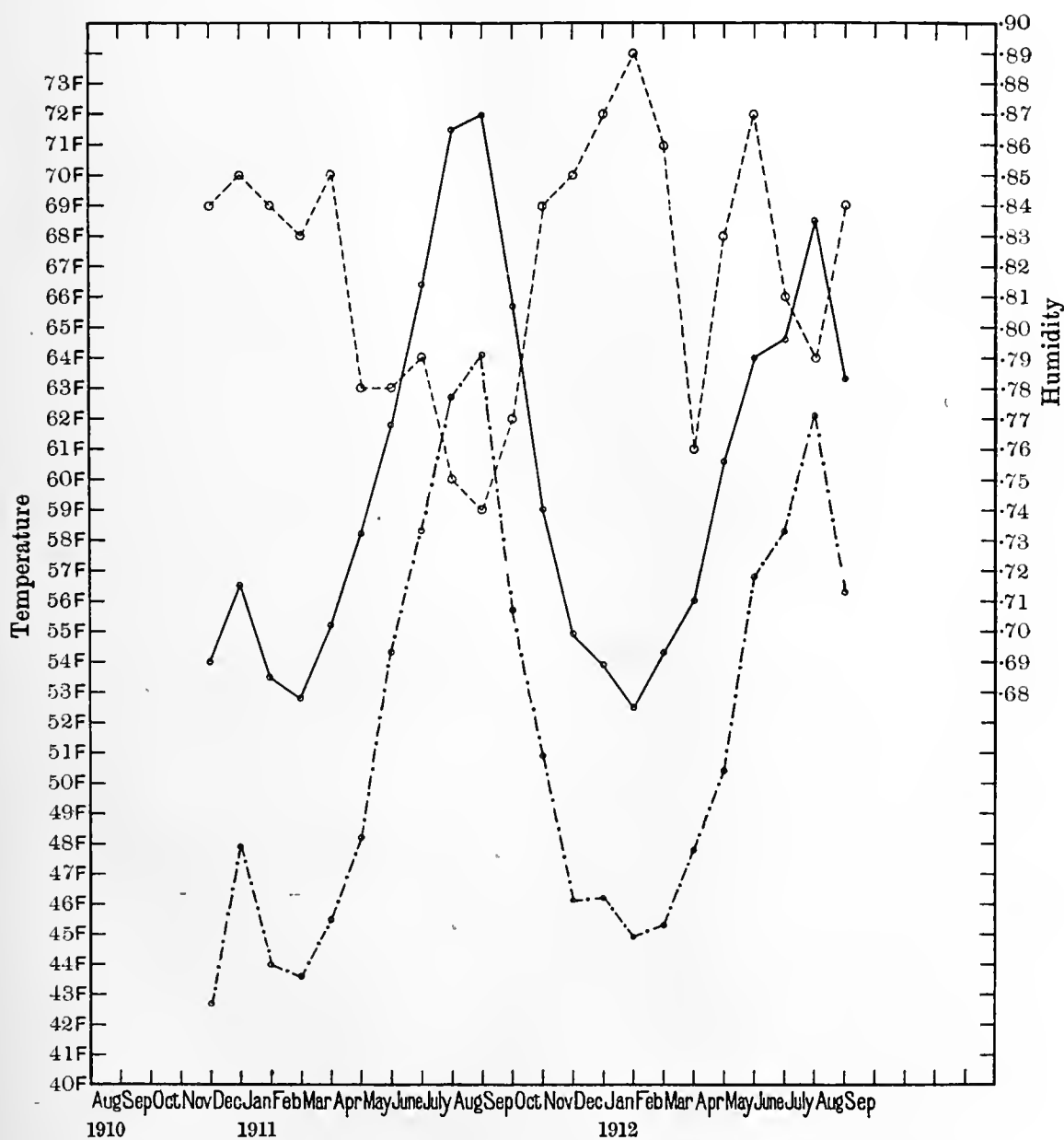


Chart 3.

Laboratory Cupboard. Temperature Max. ——— Humidity ———  
Min. - - - - -

Full records of temperature and humidity were not kept for the cupboards and cellar until the autumn of 1910; there is also a gap from April to July 1912, when the readings of "Wet" and "Dry" bulb thermometers were not taken in the cellar, as the latter instrument was in use elsewhere. The previous record, however, over a long period, and that for the months of August and September 1912, had remained constant so that the estimate of '93 is considered near enough for all practical purposes.

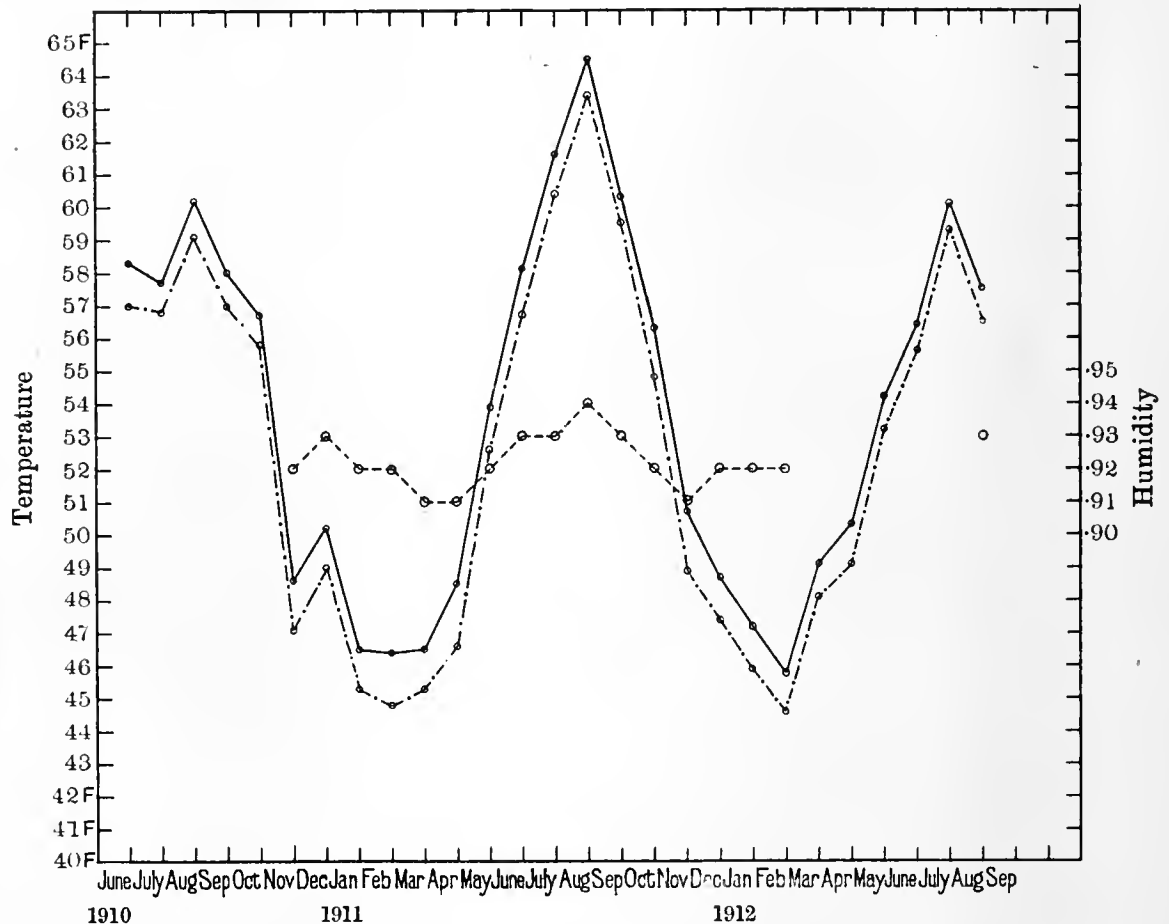


Chart 4.

Cellar. Temperature Max. ————— Humidity -----  
 Min. -.-.-.-.-

**BEEHIVE.** (Chart No. 6.) This was installed as a control to test the influence of the natural extremes of night and day temperatures, chiefly with regard to the cocoon stage. No humidity records were taken as it was thought that these were unlikely to yield trustworthy comparison with the conditions elsewhere unless taken at frequent intervals. As might be expected, the results obtained by its use varied according to the weather conditions prevailing, but evidence is afforded that

the larvae of some species can withstand considerable cold, even when newly hatched.

In order that the records of humidity might be as accurate and reliable as possible, Dr Martin kindly devised an air circuit past the wet bulb thermometers in the incubators in order to guard against the

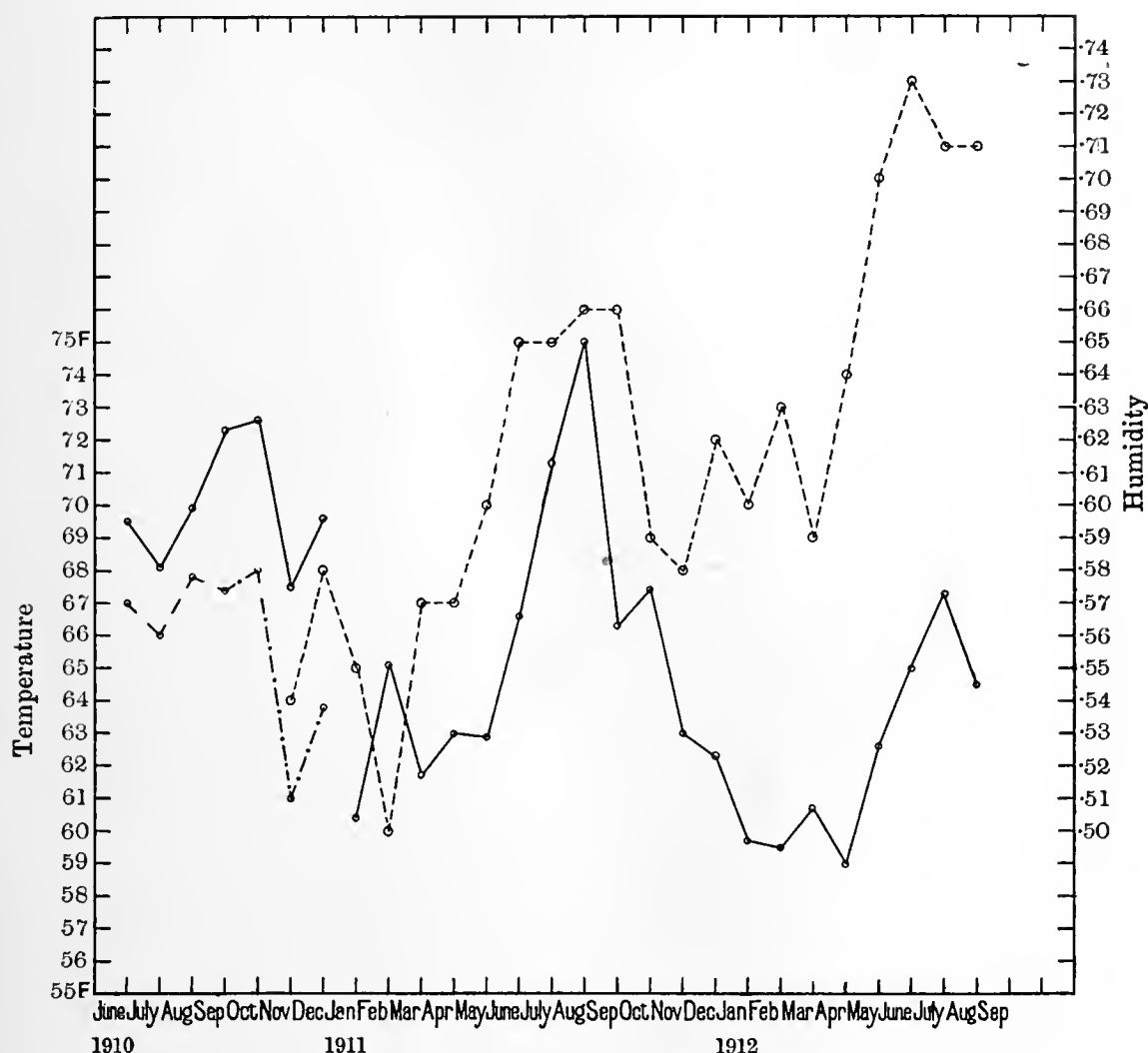


Chart 5.

Warm Cupboard. Note up to Dec. 1910 Max. and Min. records were made, after that date readings are of the "dry" bulb of the Hygrometer.

Temperature Max. ——— After Dec. 1910 Dry bulb of Hygrometer ———  
Min. - - - - - Humidity - - - - -

error involved when readings are taken in a nearly still atmosphere. The readings were in every case the minimum obtained after working the air circuit illustrated on p. 459. There still remains, however, the fact that the mortality in such a place as a house cupboard is far higher in comparison than in the incubators. It is suggested that this is

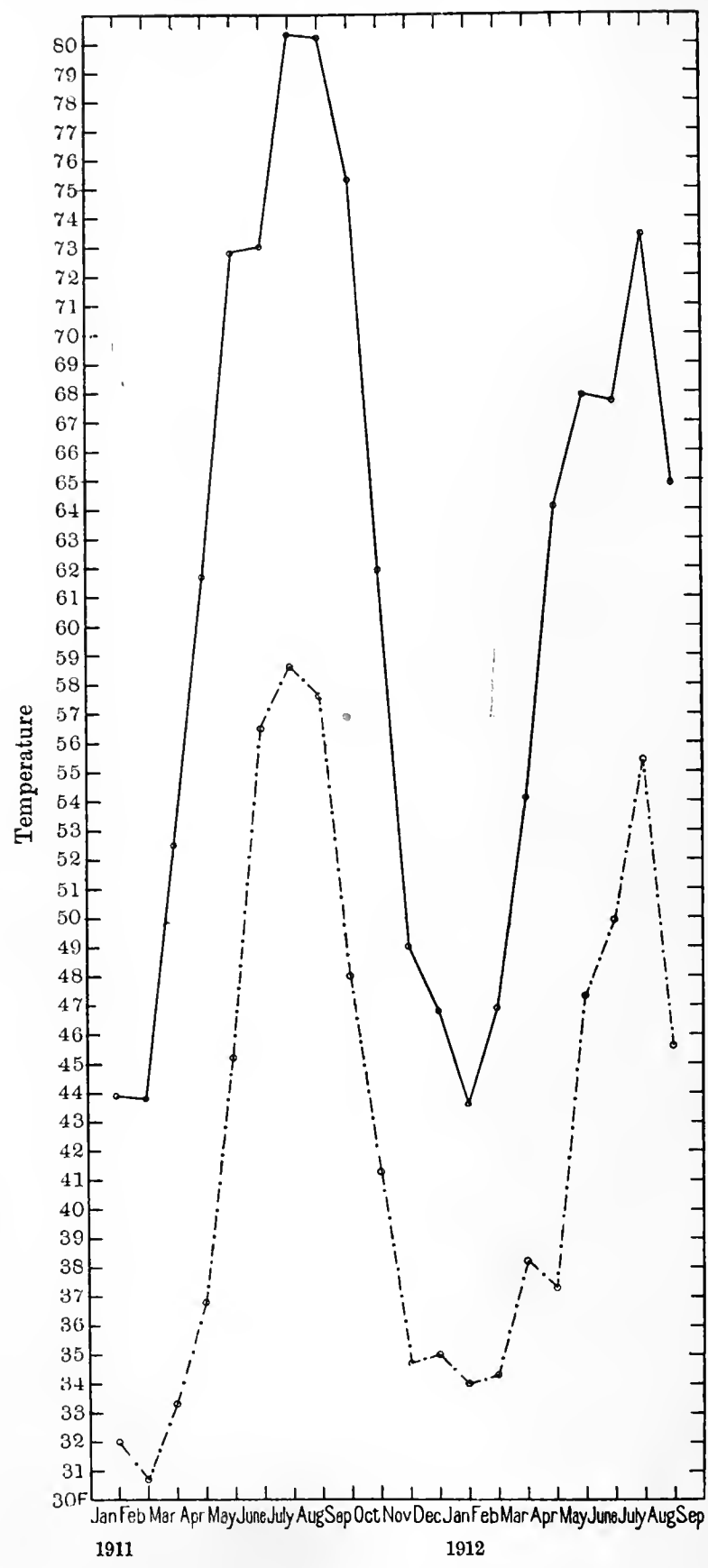


Chart 6.

Beehive. Temperature Max. — Min. - - - - -



due to the inequality of the humidity in the latter. The small amount of current in an incubator possibly allows of "pockets" of moist air to remain undisturbed in the receptacles in which the larvae are reared. Possibly the moisture given off by the larvae themselves may produce an envelope of humid air which enables them to escape the desiccation that would inevitably occur in a more draughty situation<sup>1</sup>.

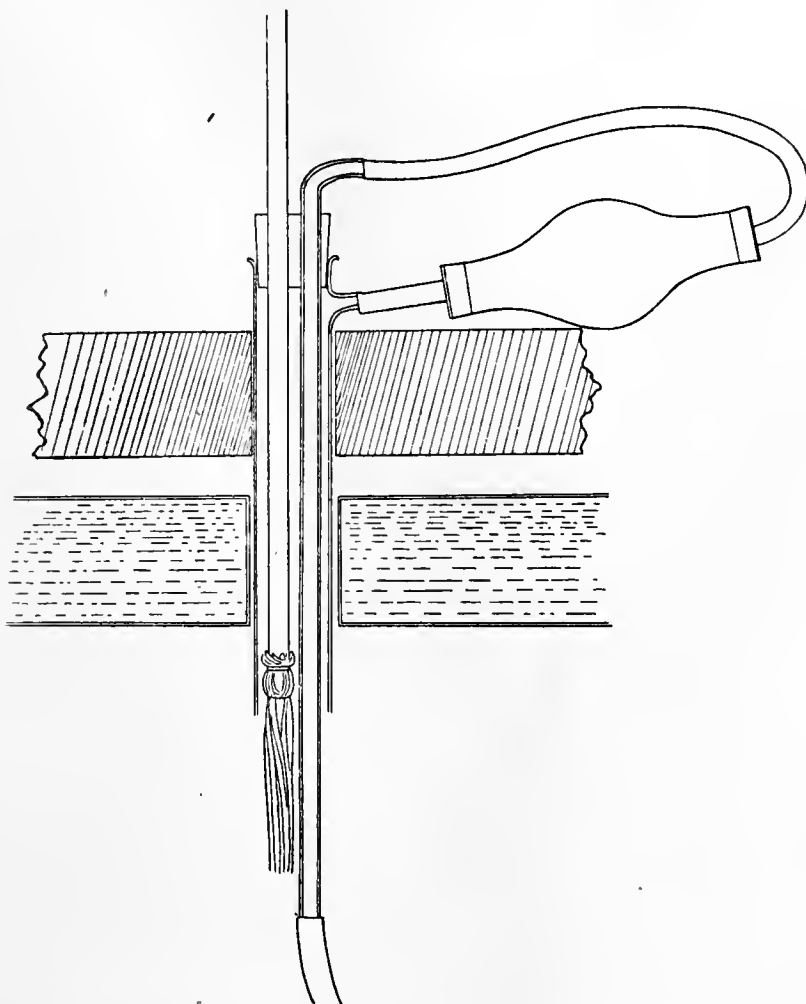


Fig. 1.

In contrasting results this possibility should be borne in mind, as it seems possible that all the incubator humidities should be regarded as higher than the actual readings, in comparison with other situations. Even with incubators having a wide range of ventilation adjustment and a water tray subdivided so as to allow of a varying area of exposed

<sup>1</sup> For example, in the case of the newly hatched larvae trials in incubator 75 Wet (see Table X); the average length of life is only half as long in July as during the cold months. This is apparently due to a less stagnant atmosphere in the former case. It is usual to keep the ventilator more widely open during the summer as this can be done without any corresponding fall in humidity.

water surface, it is impossible, during the summer, to maintain a steady percentage of moisture in the air for more than a few hours or days together, as all the conditions are swayed by external changes. In the winter and autumn, especially at night, when a clearing sky results in a ground frost, there is a rapid fall in the amount of moisture in the outside air drawn into the incubators, and when hard frosts set in it is difficult to maintain sufficient humidity to enable the larvae to escape destruction, even with the ventilators shut down and wet cloths hung in the interiors.

*Incubators. Monthly Averages of Humidity and Temperature.*

Month 1910	Incubator 85° Wet Temp.	Hum.	Incubator 75° Wet Temp.	Hum.	Incubator 85° Dry Temp.	Hum.	Incubator 75° Dry Temp.	Hum.
Aug.	84.1	.72	74.3	.68				
Sept.	84.8	.70	74.6	.65				
Oct.	84.2	.71	74.3	.74				
Nov.	85.9	.71	74.2	.73	84.3	.43	74.7	.49
Dec.	84.3	.72	74.4	.77	84.1	.57	75.3	.50
1911								
Jan.	84.7	.67	74.3	.75	85.0	.56	76.4	.55
Feb.	84.1	.69	74.4	.75	84.2	.60	75.9	.49
March	83.7	.71	74.3	.76	84.1	.59	75.4	.49
April	84.0	.72	75.0	.78	83.8	.61	75.6	.47
May	84.0	.76	74.9	.85	84.0	.60	75.1	.51
June	84.0	.80	75.8	.86	83.9	.60	75.2	.53
July	85.1	.83	76.3	.85	84.3	.64	76.3	.57
Aug.	84.7	.81	75.0	.80	83.8	.63	75.7	.59
Sept.	84.8	.77	74.8	.76	84.4	.59	74.9	.54
Oct.	84.5	.78	74.4	.73	84.7	.60	74.6	.52
Nov.	85.2	.73	75.0	.69	92.9	.56	74.4	.54
Dec.	92.4	.66	75.8	.65	92.8	.57	74.7	.51
1912								
Jan.	92.6	.66	75.5	.71	93.2	.56	75.2	.50
Feb.	92.4	.68	75.0	.76	93.0	.60	73.5	.56
March	92.3	.67	75.1	.79	93.1	.60	73.3	.56
April	93.1	.69	75.8	.79	93.7	.61	74.1	.56
May	95.1	.75	75.6	.86	93.3	.58	74.7	.59
June	94.5	.77	74.9	.83	93.0	.55	75.8	.54
July	95.2	.74	75.0	.86	93.0	.57	76.2	.64
Aug.	94.2	.75	74.3	.81	93.0	.54	75.0	.56
Sept.								

(Readings twice daily.)

NOTE. A 3 Nov. 1910 20 Temp. and 14 Humidity readings only.  
F 3        „        20        „        14        „        „        „

*Laboratory Cupboard.*

	Number of readings	Temperature		Humidity
		Max.	Min.	
1910				
Nov.	23	54.0	42.7	.84
Dec.	31	56.5	47.9	.85
1911				
Jan.	31	53.5	44.0	.84
Feb.	28	52.8	43.6	.83
March	31	55.2	45.5	.85
April	30	58.2	48.2	.78
May	31	61.8	54.3	.78
June	30	66.4	58.3	.79
July	31	71.5	62.7	.75
Aug.	31	72.0	64.1	.74
Sept.	30	65.7	55.7	.77
Oct.	31	59.0	50.9	.84
Nov.	30	54.9	46.1	.85
Dec.	31	53.9	46.2	.87
1912				
Jan.	31	52.5	44.9	.89
Feb.	29	54.4	45.3	.86
March	31	56.0	47.8	.76
April	30	60.6	50.4	.83
May	31	64.0	56.8	.87
June	30	64.6	58.3	.81
July	31	68.5	62.1	.79
Aug.	31	63.3	56.3	.84
Sept.				

*Cellar.*

	Number of readings	Temperature		Humidity
		Max.	Min.	
1910				
May	19	56.0	54.1	
June	28	58.3	56.9	
July	31	57.7	56.8	
Aug.	30	60.2	59.1	
Sept.	30	58.0	57.0	
Oct.	30	56.7	55.8	
Nov.	30	48.6	47.1	.92 (27 readings of humidity)
Dec.	30	50.2	49.0	.93
1911				
Jan.	31	46.5	45.3	.92
Feb.	28	46.4	44.8	.92
March	31	46.5	45.3	.91
April	30	48.5	46.6	.91
May	31	53.9	52.6	.92
June	30	58.1	56.7	.93
July	31	61.6	60.4	.93
Aug.	31	64.5	63.4	.94
Sept.	30	60.3	59.5	.93
Oct.	31	56.3	54.8	.92
Nov.	30	50.7	48.9	.91
Dec.	31	48.7	47.4	.92
1912				
Jan.	31	47.2	45.9	.92
Feb.	29	45.8	44.6	.92
March	31	49.1	48.1	.93 (estimated)
April	30	50.3	49.1	.93 (estimated)
May	31	54.2	53.2	.93 (estimated)
June	30	56.4	55.6	.93 (estimated)
July	31	60.1	59.3	.93 (estimated)
Aug.	31	57.5	56.5	.93 (27 readings of humidity)
Sept.				

*Bionomics of Fleas**Warm Cupboard.*

1910	Number of readings	Temperature		Humidity
		Max.	Min.	
May	18	71.9	67.1	
June	28	69.5	67.0	
July	31	68.1	66.0	
Aug.	31	69.9	67.8	
Sept.	30	72.3	67.4	
Oct.	30	72.6	68.0	
Nov.	30	67.5	61.0	.54 (27 readings of humidity)
Dec.	28	69.6	63.8	.58 (31 readings of humidity)
1911		Dry Bulb		
Jan.	29	60.4		.55
Feb.	27	65.1		.50
March	31	61.7		.57
April	29	63.0		.57
May	30	62.9		.60
June	30	66.6		.65
July	28	71.3		.65
Aug.	30	75.0		.66
Sept.	30	66.3		.66
Oct.	29	67.4		.59
Nov.	30	63.0		.58
Dec.	30	62.3		.62
1912				
Jan.	31	59.7		.60
Feb.	29	59.5		.63
March	31	60.7		.59
April	30	59.0		.64
May	31	62.6		.70
June	30	65.0		.73
July	31	67.3		.71
Aug.	31	64.5		.71
Sept.				

*Beehive.*

1910	Number of readings	Temperature	
		Max.	Min.
Dec.	3	50.0	29.0
1911			
Jan.	31	43.9	32.0
Feb.	28	43.8	30.7
March	31	52.5	33.3
April	30	61.7	36.8
May	31	72.8	45.2
June	30	73.0	56.5
July	30	80.3	58.6
Aug.	31	80.2	57.6
Sept.	30	75.3	48.0
Oct.	31	61.9	41.3
Nov.	30	49.0	34.7
Dec.	31	46.8	35.0
1912			
Jan.	31	43.6	34.0
Feb.	29	46.9	34.3
March	31	54.1	38.2
April	30	64.1	37.3
May	31	67.9	47.3
June	30	67.7	49.9
July	31	73.4	55.4
Aug.	31	64.9	45.6
Sept.			

(b) *General arrangement and management of breeding cages.*

For rearing rat fleas I have used a slightly modified form of the cage designed for the infection experiments of the Advisory Committee illustrated in the *Journal of Hygiene*, Vol. VI. No. 4, Sept. 1906, Plate IV. The alterations consist of somewhat enlarging the cage and making the inner cage larger in proportion to the outer. Several different sizes have been tried, the most convenient is: Outer glass cage 10" × 10" × 18", inner wire cage 8" × 8" × 8", neck or entrance tube 5" in diameter by 3" deep. The pan 9" × 9" × 1" deep. Copper or brass wire gauze is preferable to iron as it does not rust. In place of the tin lid a wire gauze cover keeps the cage drier and sweeter, and a muslin cover<sup>1</sup> may be used over this to ensure that no fleas escape. The cover of the outer cage is of muslin tied round the neck or entrance tube and fastened to the glass sides with glue. It is well to use some cement that can be softened, as portions of the cover have to be removed occasionally. The floor, pan and all, should be covered with about one to one and a half inches of silver sand or sawdust. Sand should be washed and then baked to make certain that it neither contains any salt nor harbours mites or other harmful organisms.

A single large rat, or two small ones, may be kept in a cage of this size without its getting too humid under normal atmospheric conditions. Occasionally, say at monthly intervals, a quantity of the sand and the rats' bedding should be shifted to the space between the wire cage and the glass and returned the following month. Under exceptional circumstances it is necessary to remove a portion of the sand and bedding entirely, in order that it may be slowly dried in some deep walled receptacle; it may then be returned with its fauna of larvae and fleas.

In cages for breeding *C. fasciatus* it is well to supply the rat with some pieces of cloth or felt as bedding; this very much facilitates the collection of both fleas and their larvae which will be found wandering over and through the felt respectively.

As regards *X. cheopis*, cloth bedding for the rat does not offer the same advantages for obtaining fleas and their larvae. The former are usually to be found in the greatest numbers on the rat itself or in the sand, while the larvae are usually distributed through the sand and are seldom seen on the cloth. Cocoons are, however, to be obtained in numbers spun on buried or partially buried pieces of cloth placed between the outer and inner walls of the cage.

<sup>1</sup> The best material to employ is what is known by drapers as "Nainsook."

FOOD FOR THE RATS. Bread and milk, given in small pans, was the only food used in the cages during the first year. It was adopted in preference to grain, bran, etc., as a precaution against mites, which are frequently present in corn chandlers' stores. A better method adopted later is to give some oats, a piece of new bread and a drink of milk daily.

With mice I am informed that the proper diet is mixed oats and hemp seed and a small piece of bread which, after being wetted and squeezed to remove the superfluous moisture, is left in the cage for an hour or so and then removed. This method has proved quite successful for mice, but for rats more liquid is essential.

The grain and seed should be heated before use to kill off any mites or other organisms it may harbour. Although we were able to keep our cages free from mites for many months, they eventually became infected, possibly by way of the animals when changes took place. At least two species were present—the more numerous being one of the vegetable-feeding Tyroglyphidae that is very commonly associated with grain stores. The other was a species of *Cheyletus* closely allied to, if not identical with, *Cheyletus eruditus*. This mite probably lives chiefly on the vegetable feeding species, but will undoubtedly attack and destroy flea larvae, a specimen having been caught in the very act.

It is questionable, however, if this species is very harmful in the cages when the other mites are numerous, as the latter will be much easier prey for the *Cheyletus* than the frantically active flea larvae. When the flea larvae are moulting, the case may be otherwise, and, should the *Cheyletus* be present to a large extent, or the vegetable feeding species of mites be absent or present in small numbers only, serious mortality of the flea population might occur.

(c) *Methods employed in rearing fleas and experimenting with them.*

1. OBTAINING AND HATCHING EGGS. To obtain ova of *Pulex irritans*, the adults are kept in one-inch cardboard boxes with glass bottoms<sup>1</sup>,

<sup>1</sup> In each incubator or other place used for experiment a jar of sand is kept in which are buried the boxes containing fleas for egg laying or cocoons awaiting emergence and the tubes of adult fleas for longevity tests. Glass tubes containing newly hatched larvae whose length of life without food is being determined should be partially buried in the sand to prevent any condensation of moisture in the tube.

This method was instituted so as to render the conditions of the experiments on adults more natural, the habit of the flea being to hide away in crevices, corners, etc., where more equable conditions of temperature and humidity are likely to obtain than elsewhere.

The result proved satisfactory, as the mortality among the fleas put into boxes for egg laying was greatly reduced, and the average number of eggs laid was increased.

the lid replaced by a cover of fine silk gauze (chiffon) securely tied on. A loose ring of dark cloth is placed round the inside of the box to give the fleas foothold, and this also proves very convenient for the removal and counting of the eggs that are laid on it. If the card of the box is stained with methylene blue or other dark colour, the finding and counting of those eggs that are laid off the cloth are facilitated. This box is nested in one of the next larger size for security and only removed for feeding, when the gauze is placed against any convenient skin surface. One quarter of an hour per day is sufficient time for feeding, but larger numbers of eggs are laid in response to more food. The fleas must be shifted to a fresh box or the ova removed every three or four days to prevent any larvae that may be hatched escaping through the mesh of the gauze.

Rat, dog or other fleas are collected from the host or its bed and put into boxes. Up to 50 females and the due proportion of males are put into a one-inch glass-bottomed box, and for greater numbers a box of one and a quarter inch diameter is used.

The box is prepared in the same way as for *P. irritans*, but the gauze cover may be omitted (for method of boxing fleas, see p. 464). The boxes containing the fleas are buried in the sand pots mentioned in footnote on p. 464. To induce the fleas to lay freely the boxes are kept in a humid atmosphere at a temperature of 70° to 75° F. The fleas are left in the boxes for 24 hours and then returned to the cages.

It is not so necessary to bury *P. irritans* as it is *C. fasciatus* and *X. cheopis*, but in the hotter incubators or during cool weather it is advisable. *X. cheopis* does not lay freely unless the cage containing both fleas and their host is kept at about 70°—75° F.

**HATCHING.** Eggs are best left where they are laid if this is possible, for, though they can usually be moved by the use of a moistened camel's hair brush with safety, it is not always possible to avoid injuring them, as they occasionally become quite firmly attached in spite of the fact that no cement is used by the female when they are laid.

The powers of abstinence on the part of the newly hatched larvae renders it practicable to leave the hatching box without food supply; the young larvae are then collected day by day by inverting the box in the mouth of a glass tube, when, after gently tapping, they fall into the bottom of the tube. A warm humid atmosphere is best to ensure a speedy emergence from the egg. Cool conditions lower, and hot and dry ones raise, the percentage of failures without proving so absolutely fatal to the ova as they are to the larvae.

2. FOOD AND TREATMENT OF LARVAE. Glass tubes, entomological boxes and card jars have been used as receptacles for rearing larvae. Glass tubes one and a quarter to one and a half inches in diameter give good results for small numbers, but they are liable to condense moisture if the air is humid or subject to large temperature variations. Glass-bottomed boxes did not give satisfactory results and are not very convenient, as with humid conditions the cardboard lids swell and become too tight. "Mono Service" card cream jars are light, unbreakable, and being made of compressed waxed card, are not absorbent, and at the same time do not condense moisture so readily as glass. Most of the experiments were carried out in half-pint jars of this description. A cover of thin muslin, fastened by an elastic band, served to keep out dust and prevent any rapidly developed fleas from escaping without seriously checking free interchange of temperature and humidity.

Food (for a general discussion on the food of flea larvae, see Section IV, subsection 2).

In the experimental work five separate diets were tried:—

(1) Blood-soaked rag, which is first to be recommended. Any slaughter-house can supply a mutton-cloth dipped in blood, and if this be wrapped in paper and put in a dry situation it will keep indefinitely and may be cut into snippets as required. This formed the chief food used in the experiments, and is designated, for brevity, B.S. Rag.

(2) Flea faeces, in which case the gauze covers to boxes in which fleas have been fed, are very convenient provided they are well spotted and blotched with the blood and dejecta voided during and subsequent to feeding. This food, referred to as "flea faeces," although the blood ingested was human, seemed to suit *C. fasciatus*, *X. cheopis* or *P. irritans* equally well.

(3) Dead flies. A few experiments were made with *Musca domestica* and *P. irritans*. The diet proved a moderate success, but required renewing constantly, and the time expended in catching flies, together with shortage of supplies in late autumn, prevented further trials. The fact that the cocoons are very difficult to find when flies are used makes this food quite unsuitable for some sections of experimental work.

(4) Bran gave very poor results for feeding *C. fasciatus* when used in the small quantities that it is convenient to work with if close observation is required before the fleas actually emerge. It is also open to the same objection as the dead flies as regards the difficulty of finding the cocoons.



(5) Rat Faeces. These gave very poor and unsatisfactory results with *C. fasciatus*. Crushing or chopping the faeces was tried and improved matters somewhat. Larvae in some instances fed well, and the percentage of cocoons was high, but the actual numbers of *C. fasciatus* reared were small. Larvae of *P. irritans* and *X. cheopis* succeeded much better on this food, which is a decided improvement on the flies and bran although the cocoons are not easy to find. Possibly under natural conditions, when a fresh supply is continually available, rat faeces may be a more important article of diet than the experimental evidence suggests.

COVER FOR THE LARVAE. A small quantity of fine sand or other finely divided material should be placed at the bottom of the receptacles used for rearing larvae; about  $\frac{3}{4}$  of an inch is sufficient, the food being mixed in with the sand. It is not necessary to use sand with bran, dead flies, or some other foods, and larvae have been reared on dried blood alone in a glass tube. Its use is, however, a necessity when rearing in a cage, as pointed out in the Reports of the Plague Commission (*Journal of Hygiene*, Vol. VIII. Chap. XXIX. Section 1), and is a convenience when rearing apart from the host, as it renders the finding of cocoons and their removal much easier. The sand or other material used ought always to be dry enough to fall grain by grain; it is detrimental when wet enough to cling.

HUMIDITY. When rearing in very dry places a local humidity may be attained by placing at the bottom of the receptacle a disc of blotting-paper with a tag projecting above the sand; this tag can be wetted at intervals. The method of placing damp blotting-paper *above* the sand or a wet muslin cover to the tube or jar may be pursued. The sand itself should on no account be damped. The adjustment of this moistening, having regard to the local conditions of temperature, humidity and air circulation, makes all the difference between 100% mortality in the first two days and the successful rearing of adult fleas, but too much or too little moisture may be equally fatal (compare tables and note the effect of moistening in incubator 75 Dry and 75 Wet, also between 85 Dry and 85 Wet).

Urine, as compared with distilled water, appears to be a more effective damping agent. Possibly the salts it contains retain the moisture and develop a pocket of moist air in the receptacle used, while with distilled water the desiccation is more complete. While admitting that the evidence for this last opinion is none too clear, there seems no doubt whatever as to the main issue of the beneficial effects of moistening generally in a dry atmosphere.

**METHODS OF HANDLING.** To collect individual larvae from the dust and rubbish of an animal's bed or nest is a slow task. By using a series of wire or muslin sieves the process may be much quickened; an ordinary wire document basket makes an excellent sieve for the first stage; small boxes with the bottoms replaced by fine mosquito netting and fine veil netting can then be used, only fine dust, larvae and eggs will be left in the pan. Much of the fine dust can afterwards be gently blown away. The best method of collecting larvae from dust or sand that is too heavy to be disposed of by winnowing is to spread the sand or dust thinly over a black or dark-coloured card and pick the larvae out with a fine moistened sable or camel's hair brush. Flea-larvae are usually very conspicuous, for such small objects, owing to their frantic activity, but occasionally may be missed as they lie still in a stiff stretched-out position or coiled up watch-spring fashion. Under these circumstances they are difficult to see, especially when small and coiled. It is best to leave the sand undisturbed for a few minutes and then re-examine, as they usually renew their activity after a short rest.

To test the length of life without food of freshly hatched larvae they were placed in small corked glass tubes, three-eighths of an inch by two inches, with a few scraps of blotting-paper to afford them foothold and cover. Coloured blotting-paper renders observation and counting easier. A subsequent method was to use a little fine sand and to plug the tube with cotton-wool; under these circumstances it is necessary to keep the tube upright.

3. **OBTAINING AND TREATMENT OF COCOON STAGE.** In treating the cocoon as a definite stage, convenience alone has been followed.

To obtain cocoons for experiment, full sized larvae are sorted out from the cages, breeding jars or the host's bed as the case may be. They are placed in a receptacle containing fine sand or dust and a supply of food and kept in a humid atmosphere at 70°—75° F. The cocoons must be removed daily from the receptacle if their subsequent history is to be at all closely followed; they are therefore removed to a one-inch glass-bottomed box and buried in the sand pots. For as the cocoons are naturally buried beneath dust or litter, or spun in crevices, it is an obvious precaution to bury the box in which they are kept if fair results are to be obtained.

4. **CONTROL AND TREATMENT OF ADULTS. TRANSFERENCE OF FLEAS.** For shifting, transferring or sorting fleas the use of a large pan with a white glazed surface, as recommended in the report of

the Advisory Committee (*Journal of Hygiene*, Vol. VIII. No. 2, p. 256, May 1908), was adopted.

For dealing with *P. irritans* a much deeper pan is necessary than for the rat fleas; it must also be wide, as otherwise the hands and arms of the operator prevent a clear view of the bottom being obtained. An enamelled iron "chef" bowl is suitable, but it should be ten inches to a foot deep, for *P. irritans* can jump at least a foot even from the slippery take-off afforded by an enamelled pan, and experience has shown that it can jump on to the clothing of a person stooping over the pan although unable to clear the rim. *Ct. canis* and *C. gallinae* are also good jumpers and require a deeper pan than either of the rat fleas. The jumping habit soon falls into abeyance, however, when the fleas are kept in small boxes and fed.

All jars, tubes or boxes in which larvae are being reared or cocoons kept, should be opened after being placed on the bottom of the pan, and in changing from box to box the same precaution ought to be followed. To collect fleas from the pan, an ordinary test-tube and a small camel's hair brush are all that is required. If the test-tube be held with its mouth in front of the flea, tilted at a slight angle so that the upper rim is above the insect, it will usually hop into the tube; the brush may be used to expedite matters or to aid refractory specimens to take a right direction. In case it is desired to collect *P. irritans* in boxes, the fleas being already in the pan, the box with cloth lining ready for their reception is dropped mouth downwards over first one and then another; each flea covered usually crawls or jumps on to the cloth lining without delay, so that boxing is not a lengthy process. When the required number are covered, a piece of fine silk gauze (chiffon) is placed over the upturned lid of the box at the bottom of the pan. The box is quickly lifted, fitted over its own inverted lid and gently forced down so that it does not quite shut. Thread can then be tied round the crack between the shoulder of the box and the lid. The lid may be removed, leaving the fleas securely imprisoned in a box with a glass bottom and a gauze cover in place of, or in addition to the lid. The process is somewhat easier if a loose fitting lid is kept specially for the process of fitting the gauze.

A more rapid method of boxing, useful for example in egg-laying experiments, is to first collect the required number of fleas in a tube and then to place in the pan a box prepared with cloth lining as described above. The lid of the box is slightly lifted and the

fleas are shot in by a rapid tilt of the tube and the lid closed immediately. With most fleas a little practice makes one deft enough to perform the operation so that few, if any, have time to escape. In the case of *P. irritans*, however, this frequently happens.

The method of storing in gauze-covered boxes described above as specially suitable in case of *P. irritans* is applicable for dealing with any species of flea that it is desired to test in regard to its aptitude to feed on man. It can also be adopted for feeding tests on other mammals, provided that their fur is closely cropped on the area of application. Feeding fleas from boxes is not without its advantage in other ways than that of convenience, for there is no danger of their escape or destruction by the host while feeding, a point of considerable importance if the number of specimens is small.

It has not been found possible to feed captive fleas on either the domestic cat or dog with the time at our disposal for this purpose. The dog was willing, but too anxious to play, while the cats were indignantly and successfully hostile.

With a tame rat the matter was easy and it was found quite unnecessary to secure the rat. By holding it beneath the left hand, head to wrist, with the two forefingers along its back and the box containing the fleas held with the right hand against the clipped patch above the tail, it was possible to give undisturbed feeds of from five to ten minutes and repeat as desired.

To test the length of life of fleas unfed under conditions as favourable as possible, the adults are put into small paper tubes, made of filter paper rolled round a penholder and secured with thread tied round them. The ends of these tubes are carefully blocked with plugs of cotton wool wrapped in fine gauze; the gauze is necessary to prevent the fleas from being lost in the plug. The tubes are buried in the sand pots previously described and kept in the places where the experiment is to be made.

A second method is simply to place the fleas in a small card jar with sand at the bottom; the fleas burrow in the sand and some species appear able to survive longer under these conditions than in tubes, for example, one specimen of *C. fasciatus* lived 95 days and one of *C. gallinae* 127 days. *X. cheopis* is also favoured by this method, but to *Ct. canis* and *P. irritans* it is of no advantage.

SECTION IV. GENERAL OBSERVATIONS ON BIONOMICS OF FLEAS.

1. EGGS (Plates XXX and XXXI). As might be expected, the general effect of cold or cool conditions is to slow down or prevent egg laying. In case of both *P. irritans* and *C. fasciatus* however it is possible to induce them to lay at any time by changing them in a warm moist atmosphere (about 75° F., humidity about 70). While *P. irritans* is the more responsive of the two, in neither case do the number of eggs laid under these conditions reach the summer average.

With *X. cheopis* the response to incubator treatment during the winter months is but slight; very few ova are obtained by removing females from the cages and placing them in an incubator at 75° F. for 24 hours. It appears necessary for egg production by this species that their host should be living in a warm atmosphere.

*Ct. canis* certainly lays under suitable conditions apart from season, as I have taken larvae from a dog's bed, indoors, as late as December.

*C. gallinae* is possibly more fixed in seasonable habit than the other species as regards egg laying. I have fed a number during the autumn months on myself, but, although kept constantly at a temperature of 75° F. except when feeding, no eggs were laid until spring.

2. LARVAE (Plates XXXI and XXXII). Rösel von Rosenhof reared fleas from the egg more than 150 years ago, and found that the larvae did not, as some supposed, feed on rotten wood. He discovered that they would feed on the bodies of their dead parents and could be reared on dead flies. The imminent failure of this source of diet with the oncoming of winter caused him to seek for another food. He made trial of the blood of some pet doves and proved that the larvae could be successfully reared on this food. No doubt—as pointed out in Report XXIX. Section 1. *Journal of Hygiene*, Vol. VIII. No. 2, May 1908, pp. 236 and 237—fleas may be reared on many kinds of organic matter if in a state of sufficiently fine division and fairly dry. The experience gained in the course of the present work, however, suggests that Rösel was very near to giving them their correct food and that the mainstay of the larval diet is the faeces of the adult fleas.

It may be that the necessity for this diet in case of larvae of a particular species depends upon the closeness of the association between the parent and the host fed upon. Fleas being chiefly, if not exclusively, nest or lair parasites, it is not surprising to find that the larvae should utilise as food the rich store of organic matter in suitable condition for assimilation that is afforded by the droppings of their parents.

It is an interesting speculation as to how far the adult's habit of wasteful feeding is the direct outcome of selective action, making a special provision for the larval food supply<sup>1</sup>. The habit of sucking to such excess that some of the intake has to be voided is not restricted to fleas; instances are recorded of species of the Hymenoptera and Lepidoptera acting in the same way.

The other conditions provided by the nest or lair of the host in nature are also ideal for the flea larvae, or, to put it in other words, the larval requirements are adjusted to those that are most likely to obtain where the eggs are dropped. The necessary conditions of warmth and humidity are provided by the host's body, while the provision of bedding and careful choice of a dry situation all fit in with the needs of the larval stage of the parasite. When the host leaves its nest or lair the temperature and humidity fall together, but, so far as observation goes, a fall in temperature will only have the effect of slowing development; a low humidity, however, if prolonged, will be fatal even when accompanied by low temperature.

*P. irritans* would appear to have diverged from the other nest-breeding fleas in respect of the sensitiveness of its larvae to external conditions; possibly the progressive civilisation of its host has forced it to become more adaptable. Larvae of this species were successfully reared under circumstances that proved fatal to *C. fasciatus* (see Tables XVI and XXI), and, for example, were able to feed satisfactorily on crushed rat faeces when the larvae of the latter species failed. Probably the trend of selective action has been in the direction of producing a race of *P. irritans* able to feed on any possible rubbish in out-of-the-way corners. Undisturbed breeding places in such immediate vicinity to its host as to receive any appreciable quantity of the parental faeces would become gradually rarer as cleanliness and comfort succeeded to the crowding and filth of primitive conditions.

Experiments carried out with *P. irritans*, *X. cheopis*, *C. gallinae* and *Ct. canis* show that there are three larval instars, two of the three moults taking place while the larvae are in the active stage—the third skin being cast within the cocoon during the metamorphosis to the pupal condition. So far the number of individuals of each species

<sup>1</sup> It is very noticeable to what an extent sick animals are attacked by fleas; after death their fur may be found full of dried faeces of these parasites, as though the fleas not only had an instinct, like that of Dugald Dalgetty in Scott's *Legend of Montrose*, for a full meal—not knowing when they might get their next—but were desirous of making sure of the future of their species by bequeathing a rich store of food for their larvae.

ollowed up is small, and it is not possible to say if any variation in the number of larval instars occurs. It seemed quite possible that the very small males of *X. cheopis*, which are not infrequent, might have one instar less, but no evidence of this has been forthcoming.

The heads of several larvae in each stage were measured and gave the following approximate ratios :

<i>Ct. canis</i>	1st	·16 mm.	<i>C. gallinae</i>	1st	·16 mm.
	2nd	·18 mm.		2nd	—
	3rd	·22 mm.		3rd	·28 mm.
<i>X. cheopis</i>	1st	·14 mm.	<i>P. irritans</i>	1st	·18 mm.
		·16 mm.		2nd	·24 mm.
		·20 mm.		3rd	·28 mm.

When reared in cages, the situation chosen by the larvae for spinning varies considerably between *C. fasciatus* and *X. cheopis*. Those of *X. cheopis* may be found like those of *L. musculi* spun on fragments of cloth, etc. given to the host for bedding. With *C. fasciatus* the cocoons are chiefly in the sand or at the bottom of the cages, seldom or never on the cloth.

3. COCOONS (Plates XXXIII and XXXIV). The cocoon is an oval envelope of silk (in the case of *C. fasciatus* which may often be more aptly described as of cement), spun by the larva, its exterior being covered with sand, dust, or any small fragments of dry material that are available. These fragments are not worked into the fabric, and it is probable that the larva collects them and attaches them together with a few silk threads and then proceeds to thicken the interior of this loose case with further accretions of gummy silk from the inside. Not infrequently the cocoon will be spun against the side or bottom of the rearing receptacle, the larger particles of sand, etc. being utilised for choice.

The cocoon of *C. fasciatus* varies from a flimsy structure composed of a few silk threads holding together grains of sand or other material, after the fashion of a string bag, to a hard, strong case, in the manufacture of which a much greater quantity of silk must be used. This forms a dense, firm lining of a yellow or brownish colour to the cocoon, which in extreme cases is almost like thin horn. Cocoons of *C. fasciatus* are easier to open than those of the other species dealt with, owing to the brittle character of the silk used.

*P. irritans* spins a quantity of soft white silk when well fed, and its normal cocoon is tough and soft.



The cocoons of *X. cheopis* are tougher and softer than those of *C. fasciatus*, and have more in common with *P. irritans* in the matter of shape as well as of texture.

Cocoons of *Ct. canis* are in appearance and texture nearer to those of *P. irritans* than to those of *C. fasciatus*. Those of *C. gallinae* have little resemblance outwardly to the cocoons already mentioned, being for the most part of pure silk, strong, soft and dense, pale brown in tint, and just transparent enough to allow of a decision as to their being empty or occupied. They are fastened to and spun amongst the material of which the nest is formed although the sand, etc. is not so firmly adherent to the fabric of the cocoon as in the case of the other species reared. The larvae of *C. gallinae* will, however, in sand produce cocoons very similar in appearance to those of *C. fasciatus* although not so hard.

Within these cocoons the larva lies doubled into a loop with some space to spare, although the acute bend in the body suggests discomfort; after pupation there is considerable space unoccupied. There is some doubt whether in the different species the extensive periods of rest which occur within the cocoon are larval or imaginal. The pupal period appears to be comparatively short, not more than a few days, so far as it has been possible to ascertain, and it seems probable that, once the pupal state is entered into, it must be carried to a conclusion without interruption. It was at first thought that the removal of pupae from their cocoons would be necessarily fatal, but this does not seem to be the case<sup>1</sup>. Resting larvae of *C. fasciatus*, as well as pupae of this and other species, have been taken from their cocoons and subsequently produced normal imagines. It is, however, likely that the normal course of development may be thwarted and a tendency to further rest be curtailed by removal, and it is improbable that such disturbed larvae could resist dry or otherwise unfavourable conditions. Certainly no instances of any continued delay in pupation have occurred in case of artificially opened cocoons.

**RESTING COCOONS OF *C. fasciatus*.** There seems good reason to think that the hard, strong types of cocoon commonly made by larvae of *C. fasciatus* are associated with the aestivating—hibernating or resting—habit; all grades, including the extreme, may be present in the same batch reared under precisely similar conditions. In some batches the resting tendency was found to be noticeably stronger than in others (Note November 1911, in the monthly cocoon series, *C. fasciatus* (Table

<sup>1</sup> In a few instances naked pupae have been found in the cages; probably the larvae had been disturbed whilst spinning.



XXXIII)). While the evidence is not conclusive that these hard cocoons are always associated with lying over, and the frail ones with prompt emergence, still in a majority of cases this would seem to be the position of affairs (see Experiment with Hard and Soft Cocoons). It is easy to understand how desiccation may be long withstood in the impervious horn-like envelopes. The chances of injury and attack, either by parasites or predatory insects, is certainly lessened by the strength of the cocoons, although it is not easy to see that even the more flimsy cocoons are in much danger from parasites penetrating.

4. ADULTS (Plates XXVII–XXIX and XXXI). EMERGENCE FROM COCOONS. All the species dealt with so far have the habit of clinging to a cocoon after emergence and the flea may be passed over as it will sometimes remain quiescent, even after shaking the tube or box in which the cocoons are kept. On the other hand, any disturbance, however slight may be followed by emergence. It is also a fact that, when cocoons are opened for examination, they are found to contain living fleas far too often for it to be reasonably supposed that they are all chance occasions in which opening has happened to coincide with emergence. This supports the conclusion that the fleas are normally in the habit of resting, at any rate for a short period and possibly for many days, before emergence, unless some disturbance gives warning of the approach of a possible host.

*P. irritans* has the habit of shamming death, by lying on its side with the legs drawn up close to its body. It was at first thought that this habit was confined to the human flea, but continued observation has proved that the other species dealt with share the habit, with the exception of *X. cheopis* only, in which it has not yet been observed. No doubt, as in the case of other insects, the sudden change from rapid movement to complete immobility is most puzzling to a pursuer and a most effective method of escaping danger.

DANGER FROM HOST. Observation suggests that rats, dogs and cats frequently take considerable pains to rid themselves of fleas, especially on their first assault. It is noteworthy that rat fleas do not attack indiscriminately, that is to say, they pick out special points of vantage, *X. cheopis* making for the shoulders, neck and chest or for a spot beneath the forelegs<sup>1</sup>. *C. fasciatus* has a favourite spot just above the

<sup>1</sup> It will be remembered that in Part III of the Report published in the *Journal of Hygiene*, Vol. VI, No. 4, Sept. 1906, pp. 465–6, this preference of *X. cheopis* for the cervical region was noted.

tail. A number of *C. gallinae*, however, did not show any discrimination in their attacks on a rat.

PERIODIC BREEDING. The results of cocoon experiments, see p. 534, both as regard *P. irritans* and *C. fasciatus*, afford evidence of the existence of a seasonal habit as regards the emergence of both species; and in case of *Ct. canis*, *C. gallinae*, *L. musculi* and our English stock of *X. cheopis*, the evidence is even stronger. With *Ct. canis* and *C. gallinae*, however, the habit is probably in the process of modification in the direction of continuous reproduction in response to the domesticated character of its hosts.

In nearly all insects seasonal habits are strongly ingrained, owing to the direct and powerful effects of climatic conditions on their constitution; with parasitic insects such as fleas a double adjustment is necessary, firstly in response to direct conditions, secondly in relation to the effects of seasonal changes on their hosts' habits. This latter factor must be most important in moulding flea bionomics, as the habits of flea hosts vary from a continual occupancy of lairs by some wild animals, such as bats and carnivora, to a spasmodic appropriation of convenient situations by wandering animals, such as rats. In the case of birds there is a strictly limited seasonal occupation of the old nest for two or three months only. It is also probable that, even when the home of the host is of a permanent character, and more or less in continual occupation, flea increase will be adjusted to the breeding periods of its host; it is unquestionably at these times that the best opportunity occurs for uninterrupted feeding on the part of the adult fleas, and a consequent ample food supply for their larvae.

Nomadic animals which do not possess the homing instinct are far less likely to have specific fleas allotted to them than other animals. With *P. irritans* there seems little need for any adjustment in temperate climes. With *C. fasciatus* the conditions have also become modified, but the primitive habits of this species are less likely to have suffered change than those of *P. irritans*, as only a portion of the rat population is closely enough associated with dwellings for their parasites to enjoy the full benefits of domestic life.

So far as ascertained in the course of these experiments, *C. gallinae* has a very definite breeding season during spring and early summer—the adults emerging in the spring and living through winter. Its habits are loose in other respects and it appears to have so adjusted itself as to be able to obtain sufficient nourishment for breeding purposes from the blood of its host almost as quickly as *P. irritans*. It is possible

that the exigencies of an existence begun in an *empty* nest have forced upon it the necessity of leaving no chance untried of providing for the future of its race.

BREEDING WHEN FED ON AN ALIEN HOST. In view of the fact that the species experimented with, can live for considerable periods when fed on the blood of an alien host the question of their breeding under these circumstances becomes one of considerable interest. Pairing certainly took place but no eggs were developed, so far as could be observed without dissecting specimens, certainly none were laid. This was at first thought to be due to the nature of the food alone, but it subsequently appeared to be connected with the conditions of captivity and the comparatively limited opportunities to feed.

*C. gallinae*. Among some fleas taken from a dog in November 1910, was a female specimen of *C. gallinae*; this flea was fed on a human host throughout the winter and during March laid a few ova which proved to be infertile. During February 1911 seven specimens of *C. gallinae* were taken from a deserted Tit's nest and were fed on a human host. Copulation was seen to occur among them but most of the males soon died off, the female specimens living longer, and one which survived for 41 days laid some 20 eggs during March. These eggs proved to be fertile, and larvae to the number of 16 were reared without loss, a few being used as specimens, the remainder producing adult fleas.

*C. fasciatus*. Trials lasting from 4 to 79 days (average 27), were carried out with three batches of fleas, comprising 22 individuals, but no ova were laid. A further experiment with this species, consisting of a batch of 16 fleas boxed with a tuft of rat's hair, was in progress for over a month, but no eggs resulted, although the box was kept in the humid incubator at 75° F. and the fleas were seen to copulate on several occasions. Both *C. gallinae* and *C. fasciatus* will pair before feeding, in fact shortly after emergence.

*X. cheopis*. Two batches, consisting of 19 and 15 individuals respectively, lived on a human host for from 18 to 104 days (average 63 days), but no eggs were laid.

*P. irritans*. Two attempts to breed this species when fed on rats failed to produce either eggs or brood. In one case six specimens were put into a cage with a young rat, and one individual lived for 40 days, but no larvae could be found nor did the cage produce any fleas. In a second attempt *P. irritans* was fed on the rat by the box method; the length of life was, however, very short in this case, none of the fleas

surviving more than 14 days, and it seems probable that little if any actual feeding was done. No eggs were laid. It is possible that *P. irritans* may breed to a limited extent on the dog as we occasionally rear a few individuals from among the abundant supply of flea larvae in the dog's bed, but it is probable that the ova which give rise to these larvae were laid within a day or so after the fleas were transferred to the dog's coat.

*Ct. canis*. Three batches consisting of 14 individuals, three of which were captured and the remainder bred, were fed on a human host for varying periods of from 15 to 245 days (average 71 days), but no ova were laid. A fourth batch of 20 specimens—fresh specimens being added from time to time—were boxed with a quantity of the dog's hairs placed in the box, to see if the association had any effect and fed for some months. No eggs were laid, however, although the box was kept in the humid incubator at 75° F. except during feeding times. In this case it can hardly be a question of season, for the same species was breeding successfully in the dog's bed while the experiment was in progress; this was shown by the presence of eggs.

About 60 freshly emerged specimens of *Ct. canis* were put into a cage with a young rat, none of them survived a month and no brood was discoverable, nor did any fleas emerge later in the cage. Ten specimens fed on the rat by the box method lived for varying periods of from 24 to 58 days (average 41 days), but no ova resulted.

*Ct. felis*. Only one attempt has been made with this species; five individuals comprising both sexes were fed on man for periods varying from 139 to 185 days. No eggs were laid.

Ova were successfully obtained from boxed specimens of *Ct. canis* taken from the dog, and of course ova of both *C. fasciatus* and *X. cheopis*, are freely obtained by the box method when the adult fleas are taken from the rat cages. This tends to suggest that some specific character of the blood ingested is the controlling factor in egg production; it must, however, be noted that when either *C. fasciatus* or *X. cheopis* has been fed by means of the box method on the rat no eggs have resulted. Three attempts were made with *C. fasciatus* fed on a rat by the box method; 24 individuals were used and survived from four to 106 days (average 23 days), yet no eggs were laid. A later attempt with rat's hair in the box has also proved negative; in this case 20 fleas were fed on a rat for some three weeks.

In comparison with *P. irritans*, all the other species observed are in much closer association with their hosts. They probably are

accustomed to feed in a much more leisurely manner and at more frequent intervals than the human flea. It was therefore decided to test the effect of more frequent feeding on man, and batches of *X. cheopis*, *C. fasciatus* and *Ct. canis* were fed twice instead of once a day, the second feeding being of longer duration than the usual 15 minutes.

The experiment proved partially successful; *C. fasciatus* was found to lay eggs freely and a gradually increasing stock of this species has been maintained, apart from any other source of supply. *X. cheopis* laid sparingly, but although fleas were reared from the resulting larvae and added to the adult feeding box, the experiment could not have been continued were it not that individuals reared on the rat were also added to keep up the supply of parents. *C. gallinae* proved a somewhat better layer than *X. cheopis* but stocks could not be long maintained without the addition of adult fleas from other sources.

It is of course quite improbable that *C. fasciatus* could perpetuate its race by adopting a human host, as no proper provision would be available for the larvae. *X. cheopis*, however, is more adaptable in the matter of food and this difficulty would not apply.

No success has, however, attended any attempts with *Ct. canis*. The adults of this species, like those of *X. cheopis*, practically live upon their host, and possibly practise very constant feeding. It may be that the two opportunities per day on an alien host are not sufficient and that very frequent feeding might induce *Ct. canis* to lay eggs when fed on man<sup>1</sup>.

The whole of the foregoing evidence is entirely opposed to the view that fleas can continue breeding in the absence of food for the adults.

To account for the records of large numbers of fleas existing in long deserted dwellings, or the unoccupied beds of animals, the explanation

<sup>1</sup> Later trials with this species have proved quite successful. When given opportunities of feeding on a human host, which extended to five hours daily, a considerable number of eggs were laid, all of which, however, failed to hatch. The possible feeding period was then increased to at least twelve hours per day and eggs were freely laid, a large proportion of which hatched. The resulting larvae were successfully reared and over thirty adult *Ct. canis* emerged from the cocoons spun by these larvae. There seems no obvious reason why dog fleas should require so much longer for feeding than those associated with rats or fowls, but I am inclined to think that the solution of the problem is in some way bound up with the needs of the larvae in the matter of food. The increased number of eggs laid by the twice fed *P. irritans* (see Sect. V., Influence of Food Supply on Fertility) appears to me to favour such a suggestion.

has been put forward that in the presence of ample nourishment for the larvae, generations of fleas might be reared in spite of an entire absence of any food supply for the adult fleas. It is assumed that the nutriment absorbed during larval existence suffices for egg production, as well as for the development of sperms in fleas; this is of course the case with many insects.

As stated elsewhere in this report, copulation certainly takes place among newly emerged unfed fleas. The habit has been commonly observed in most of the species dealt with and may be universal. Dissection of the larvae, pupae and imagines proves that developing ovaries may be found in the full fed and resting larvae, and onwards through the pupal period in various stages of development. The ova, however, are undersized, even after active imaginal existence has started. Throughout the whole course of these experiments oviposition has always been preceded by several days' feeding, and no single instance was observed in which an egg was laid by an unfed female; all the evidence points to the necessity of feeding the adult for the full development of eggs.

The fertility experiment conducted with adults of *P. irritans* to ascertain if length of feeding time had any result on the percentage of eggs hatching also supports this view; it was found that feeding twice a day resulted in a much larger number of eggs than a single daily meal. The number of opportunities for feeding had, however, no noticeable influence on the proportion of eggs which hatched.

When feeding the different species on man by the box method, a considerable difference was noticed in the character of the faeces deposited on the gauze and sides of the box. *P. irritans*, as was to be expected, fed to the greatest excess, blotching and splashing the sides of the box and gauze cover with semi-fluid dejecta, which were frequently bright red in colour. *Ct. canis* tends to deposit its faeces in little piles one upon the other, suggesting that the dejecta in this case were of a more pasty nature than those of the human flea. *C. gallinae* makes a deposit of neat raised dots, very similar to that of *C. fasciatus*, but the feeding of the latter species, to judge by the deposit, seems to be of a less vigorous character. The faeces of the other species tend to be darker in colour than those of *P. irritans* and I have never seen any trace of the fresh blood appearance in the former. It seems not improbable that this difference is due, not so much to a difference in digestion, as to the speed of feeding. In a few minutes *P. irritans* reaches the stage when it voids old accumulations from the gut, whereas,

in the case of the other species, the feeding period expires ere they have been able to obtain anything like a surfeit.

IRRITATION CAUSED BY FLEA BITES. The irritation caused by fleas on the human body is known to vary very widely in case of different persons. My assistant developed a distinct papular rash on his arm after feeding some *C. fasciatus* thereon, although the attacks of *P. irritans* did not in any way incommode him. Personally, the bites of fleas do not cause me any irritation whatever, although I am keenly conscious of their progress over my skin. I continued to feed upon myself the particular batch of *C. fasciatus* which caused the trouble referred to above, until they died some weeks later, but without any signs of a rash appearing. Since then I have fed numbers of individuals of the various species upon the same skin area for months together without inconvenience, and my assistant has also fed many specimens including *C. fasciatus*, for several separate periods of a week or more, while I was absent from home, without any recurrence of the rash.

A similar instance of a rash caused by *C. fasciatus* is mentioned by Chick and Martin (1911) and the question of irritation caused by rat fleas and the reaction to them is discussed briefly by Boycott (1912).

LENGTH OF LIFE. The great range of individual variability presented in the earlier stages of the fleas' existence is maintained as regards the length of the adults' life; of this the experiments give abundant examples. Some individuals die off quite early in the experiment, especially when the host is alien. The majority go steadily on for a time, dropping out gradually in a middle period. A few become thoroughly adjusted to their environment and food and far exceed all their companions in longevity. These very long lived specimens are always, in my experience, females.

#### SECTION V. EXPERIMENTAL STUDY OF THE INFLUENCE OF EXTERNAL CONDITIONS UPON THE VARIOUS STAGES IN THE LIFE HISTORY.

It will be noticed that in the earlier experiments there is fair continuity between the tables dealing with ova and those concerning the larval period (see ova Tables I, II, III, and V; larvae Tables XVI, XVII, XVIII, XXI, and XXII, up to March 1911). The cases where experiments dealing with ova were not continued through the larval stage are few and the deficiency was owing to the young larvae being required for bacteriological experiments. There are also cases in which larval experiments have no previous record. This is due to the difficulty



and great labour involved in determining the exact time and circumstances of the hatching. There is no artificial break in continuity from larval to cocoon stage in this series, but many of the larval records end before the spinning period is reached (see Tables XVI, XX and XXI).

In the experiments subsequent to March 1911, no continuity is attempted from the egg- through the larval- to the cocoon stage, all three are treated separately. The hatching experiments were concluded at the emergence of the young larvae, which were then returned to cages in which reserve stocks were kept. For the rearing experiments only those larvae were used which hatched from eggs laid and kept at 75° F.; they were removed daily and divided into batches for distribution among jars maintained under different experimental conditions. For tests with cocoons, full sized larvae were taken from the breeding cages (or breeding jars in case of *P. irritans*) and placed with food and sand at 75° F. The cocoons were then collected daily in batches and distributed to the experimental jars as in the case of larvae.

### 1. EGGS.

In the experiments made with ova before the end of January 1911, the method adopted was to place the eggs on paper or cloth in the receptacle in which the larvae were to be reared, the sand and food being added before the eggs were put in. This plan was adopted so as to give the larvae every chance of survival, but it entailed the rather cumbersome process of examining the ova individually to see if they had hatched; the periodic examination of the eggs was also rendered difficult because they were frequently dislodged or dragged off the paper or cloth into the sand, presumably by the larvae in their attempts to get clear of the shell<sup>1</sup>.

After the first experiment with newly hatched larvae had revealed their fasting powers the much more convenient plan was adopted of placing each batch of eggs in a clean dry tube and recording the number of larvae removed daily to the receptacle in which they were to be reared. A quantitative examination of the empty shells was also made in a number of cases and the results were found to be in close agreement with the record of larvae transferred. The earlier

<sup>1</sup> The egg shell is ruptured, not eaten through. A long slit on the side of the egg is made through which the larva escapes. This slit appears to give the empty shells very elastic or spring-like qualities, for they will frequently fly at the slightest touch of a camel hair brush.



method may therefore be taken to give correct results of hatching save that the number of eggs remaining on the cloth, etc. was often considerably less than the actual number of ova used and the percentage of fertility is apt to be lowered owing to the fact that fertile eggs are more apt to be dislodged than the infertile.

*C. fasciatus*. A comparison of moistened with unmoistened conditions (Tables I and II) in the incubators shows, in case of *C. fasciatus*, that moistened conditions are on the whole favourable to hatching of eggs while a slight tendency in the opposite direction is shown in case of incubator 75 Wet. The advantage of humidity is also seen by comparing results of experiments in the incubators in which hatching took place in tubes with those where jars or boxes were used. The proportion of eggs is higher in the former case presumably because the conditions were much more favourable to the retention of moisture. In the experiment made in Incubator 75 Wet the result is, however, doubtful and, in case of hatching in the cellar and laboratory cupboard, the reverse is true. The favourable effect of humid conditions in the general experiments is, as a whole, not very apparent as it is to a considerable extent masked by the wide range of individual variation which appears to apply in some measure to the fertility of eggs as well as the other characters of these insects. When, however, we select a single experiment planned on lines to bring out this point the advantage of humid conditions seems unmistakable.

*Experiments with ova of C. fasciatus in card jars (unmoistened)*  
*Table II.*

Date	Incubator	Temperature	Average humidity during progress of experiment	Number hatching
21 Dec. 1910	75 Wet	74.2 F.	.75	28 out of 32 = 87%
21 Dec. 1910	75 Dry	75.7 F.	.46	14 out of 26 = 54%
24 Dec. 1910	85 Wet	83.6 F.	.70	16 out of 26 = 62%
24 Dec. 1910	85 Dry	83.8 F.	.59	9 out of 28 = 32%

To test the matter further, a series of experiments, dealing with eggs alone, was planned to detect, if possible, the relative effect of temperature and humidity on the number of eggs laid, their fertility and the percentage hatching (see Table IV). A large number of *C. fasciatus* were taken from the cages and put together ("bulked"), they were then counted out into batches, each batch containing 50 to 70 females. The males were put in with the females, but not counted—there would be, however, approximately the same proportion of males

in each batch. Each batch was placed in a 1½" glass-bottomed box with a strip of cloth arranged to receive the eggs as described—the box being then buried in a jar of sand and placed in incubator, cupboard, cellar, etc. In series I the ova were allowed to hatch in the place in which they had been laid; in series II eight separate boxes containing eggs laid in different situations were all placed to hatch out in incubator 75 Wet, while in series III the experiment was varied by putting the boxes containing the fleas into incubator 75 Wet, bulking the ova laid, and then putting them away in batches of 50 to hatch out in different places (see Table IV). The four batches which were in incubator 75 Wet throughout (first experiment in each series), should give a measure of variability, and it will be seen that this is a wide one, viz. in the four experiments the proportion of eggs hatched varied from 71 to 85 %. The difference between the incubators 85 Dry and 75 Dry, and 85 Wet and 75 Wet, is not so marked as in the contrasted experiment referred to above under date of December 1910; this is most probably attributable to the fact that the humidity of incubator 85 Dry and 75 Dry, though still considerably lower than in 85 Wet and 75 Wet, was higher in July 1911 than in December 1910; being, for 85 Dry, .66 in July 1911, as against .59 in December 1910, and for 75 Dry, .55 as against .46. Although the effects of temperature and humidity are clearly apparent, apart from individual variability, some of the finer points that it was hoped to throw light upon are more or less submerged, or reduced to the value of possible tendencies. The results of the three series of experiments set out in Table IV may be summarized as follows, using incubator 75 Wet as a standard:

The conditions in 85 Wet are detrimental to hatching but favourable to the fertility of the eggs laid.

The conditions in 75 Dry are detrimental to hatching but favour the fertility of the eggs laid.

The conditions in 85 Dry are detrimental to hatching and perhaps to the fertility of the eggs laid as well.

The conditions in cellar are favourable to hatching but detrimental to the fertility of the eggs laid.

The conditions in warm cupboard are detrimental to hatching but favour the fertility of the eggs laid.

The conditions in laboratory cupboard are detrimental to hatching but favour the fertility of the eggs laid.

The conditions in beehive are detrimental to hatching but favour the fertility of the eggs laid.

With regard to the suggestion above, that low humidity is favourable to the fertility of the eggs laid under these conditions it must be

admitted that the facts are equally well explained on the assumption that the fertility is in inverse ratio to the number of eggs laid. High temperature when combined with a fair to high humidity is especially favourable to egg laying. It will be observed that in incubators 85 Dry and 85 Wet, which were on the whole not favourable to the fertility of the eggs laid in them, the number of eggs laid per female was usually greater than in those places which appear more favourable to fertility.

*P. irritans*. A similar experiment was planned (Table VI) on lines as nearly as possible to the foregoing one with *C. fasciatus*.

20 females, after feeding for one week, were divided into four batches and placed in boxes with a number of males which had been treated similarly. The batches A, B, C and D were fed daily for 15 to 20 minutes and kept for the rest of the day in sand pots placed in incubators 75 Wet, 75 Dry, 85 Dry, and the cellar respectively; eggs were removed from the boxes every three or four days. The females of each batch A, B, C and D were unchanged throughout and those that died were not replaced. The males, however, were replaced as they died and their numbers were increased from three to 10 in each box, but this increase was always made at the same time from a single batch that had emerged within a few days of each other, so as to preserve similar conditions for each batch, the object being to vary temperature and humidity only.

After the batches had been kept under the original set of conditions for some two or three weeks they were submitted to fresh ones and after a further period returned to their original situations. In the first two experiments of each batch the ova were allowed to hatch in the situation in which they had been laid, later they were all put into incubator 75 Wet to hatch in order that the effect of varied conditions on their fertility might be tested. Batch A started in incubator 75 Wet, was transferred to 85 Dry and returned to 75 Wet. Batch B started in incubator 75 Dry, was transferred to the cellar and returned to 75 Dry. Batch C started in incubator 85 Dry, was transferred to 75 Wet and returned to 85 Dry. Batch D started in the cellar, was transferred to incubator 75 Dry and returned to the cellar. Finally (see Table VI (a)) all the different batches of fleas A, B, C and D were kept under similar conditions, the eggs laid were "bulked," divided into batches and put away in different places to hatch.

The experiment shows that hot dry conditions were most favourable to oviposition, the results being as follows:—the adults in incubator 85 Dry (temperature 83·9° F., humidity ·61) showed 1·54 eggs per

female per day: incubator 75 Dry (temperature 74.6° F., humidity .60) 1.40: incubator 75 Wet (temperature 75° F., humidity .77) 1.11 and cellar (temperature 63° F. max., 62° F. min., humidity .94) .70 eggs per female per day.

The effect of transference from the coolest conditions to a higher temperature and drier atmosphere (batch D) was to increase egg laying, and for reverse conditions (batch B) to decrease it—both changes having a progressive effect; that is to say, in both cases the increase or decrease was more marked in the second period of time than the first. On the other hand, change from the moderate conditions of heat and moisture in incubator 75 Wet (batch A) to the hotter and drier incubator, 85 Dry, tended at first to check but subsequently to increase egg laying, while the reverse change (batch C) caused a progressive rise beyond an already high percentage. The return of the boxes to their original situations produced a rise with batch A, a reduction with batch C, a marked increase with batch B and an equally noticeable drop for batch D.

The final transfer of the batches to incubator 75 Wet produced an increase in the number of eggs laid in case of batches B and C, presumably in response to the more humid conditions; a marked rise in batch D was also to be expected. In case of batch A which had remained in incubator 75 Wet for two successive periods the number fell in the second. This may have been an oscillation in the reverse direction after its spurt following upon the previous change, or, more probably, in response to the falling humidity in incubator 75 Wet itself.

Individual variation is not so apparent in this experiment as it was in that dealing with *C. fasciatus*. This was to be expected in view of the fact that we are here dealing with only 20 females of *P. irritans*, whereas with *C. fasciatus* hundreds were taken from the cages on several different occasions.

The influence of temperature and humidity upon fertility, apart from the percentage hatching, is shown by a comparison of the percentage of eggs hatching in incubator 75 Wet after being laid under other conditions and may be studied from Table VI (b) of which the details have been collected from various experiments in Table VI. For instance, the eggs of batch B when laid in incubator 75 Dry show a higher percentage hatching in incubator 75 Wet (58%, Table VI (b)) than in incubator 75 Dry (44%, Table VI); when the ova of this batch were laid in incubator 75 Wet and placed in incubator 75 Dry to hatch, the proportion hatching was lower still. In these figures, other things being equal, we have evidence that the conditions in incubator

75 Dry are favourable for fertility of the eggs laid there, as shown by the increased proportion hatching when moved to suitable conditions for this second stage.

In Table VI (a) are given the results of a special experiment to determine the best conditions for hatching, all the eggs used being laid in incubator 75 Wet and placed in different places to hatch out. From the results it seems clear that the conditions of heat and drought in incubator 93 Dry are detrimental to hatching<sup>1</sup>, and this is also the case in incubator 75 Dry, which is still drier but not so hot; the cellar, on the other hand, while detrimental to fertility (see Table VI (b)) is favourable to hatching.

The experiments with *P. irritans* do not lend any support to the suggestion mentioned in case of *C. fasciatus*, viz. that fertility might bear some relation to egg production, being in inverse ratio to the number of eggs laid. That is to say that, under the forcing conditions of high temperature, immature eggs are deposited. With *P. irritans* it would rather seem that the high temperature induced not only free laying, but increased facility for sperms to come in contact with the eggs.

The suggestions to be gained from the above experiments as regards hatching and fertility of eggs are the following

*P. irritans. Incubator 75 Wet as Standard.*

The conditions in incubator 85 Dry favour fertility and are detrimental to hatching.

The conditions in incubator 75 Dry favour fertility and are detrimental to hatching.

The conditions in the cellar are detrimental to fertility but favourable to hatching.

*X. Cheopis.* Considerable difficulty was experienced in getting sufficient stock to carry out egg experiments with this species on as full a scale as with *C. fasciatus*. The rapid breeding that took place during the summer of 1911 led to an expectation of a liberal supply in the autumn and winter, but experience proved that the numbers dwindled rapidly when the hot weather had passed and the effects of renewed breeding were not apparent in the cages until late spring and early summer of 1912. There is no doubt that this species requires an average temperature of from 65 F. to 70 F. for rapid increase, and, quite apart from the question of seasonal habit, the temperature in the laboratory, where the cages were kept, fell so low during the night as to check breeding except during really warm summer weather.

<sup>1</sup> The continuity of this experiment with the one detailed in Table VI has been somewhat impaired by a rise in the temperature of incubator 85 Dry from 85° F. to 93° F. in order to carry out the experiments dealing with *X. cheopis*.

The methods followed for the study of external conditions upon fertility and hatching of eggs were the same as those adopted with *C. fasciatus*, which have already been described; the results are set forth in Tables VII and VIII.

From a study of the earlier experiments (see Table VII) performed during July and November 1911 and January 1912, it will be noticed that either a seasonal change takes place with regard to the fertility of the ova (*i.e.* eggs are less fertile when laid out of season), or else the reduction in humidity, which is of general occurrence in the incubators during the autumn, has a detrimental effect on their hatching. Whatever be the cause it is patent that cool conditions, such as are presented by the laboratory cupboard, cellar and beehive during autumn, winter and spring, are fatal to *all* the eggs subjected to them. The falling off in the percentage of eggs hatching of both *P. irritans* and *C. fasciatus* that are placed at temperatures in the neighbourhood of 55 F. or lower is also very marked (see *C. fasciatus* Table IV, cellar 20th April 1911, and *P. irritans* Table VI), although there is no case of the complete failure of all the eggs in any one batch.

The results of the threefold test with *X. cheopis* (Table VIII) are disappointing in view of the small number of eggs deposited, and show to an even greater extent than *C. fasciatus* the variability as regards the percentage of eggs hatching in incubator 75 Wet on different dates. This difference must be taken into account, nor can we draw any safe conclusions as to the effects of the varied conditions to which the eggs were subjected, unless the trials took place on identical dates. Under these conditions, as the eggs used were all portions of a single batch or all laid by detachments of one body of females, the difference may be taken to represent the effect of the varied condition alone.

The results, summed up in a brief table of conclusions, as in the case of *P. irritans* and *C. fasciatus*, may be stated as follows:

*Incubator 75 Wet as Standard.*

The conditions in incubator 93 Wet are unfavourable but not necessarily fatal to both fertility and hatching.

The conditions in incubator 75 Dry are on the whole somewhat less favourable to both fertility and hatching than 75 Wet but any change from one to the other is distinctly unfavourable.

The conditions in incubator 93 Dry are certainly very unfavourable, probably fatal, to hatching, but as regards the fertility of the ova laid are not unfavourable, about *parallel* with 75 Dry.

The conditions in cellar are very detrimental to egg laying and unfavourable to hatching; the effect on fertility is questionable.

The conditions in warm cupboard are somewhat unfavourable for hatching but not to the fertility of eggs laid.

The conditions in laboratory cupboard are distinctly unfavourable for laying, hatching and perhaps fertility as well.

The conditions in beehive are distinctly unfavourable for laying, hatching and perhaps fertility as well.

The conditions in ice-chest are fatal.

The evidence for these conclusions is admittedly even less decisive than it was for the tabulated conclusions in regard to the other species. They must be viewed in the light of suggestions and in no way final.

TABLE I. *Ova C. fasciatus.* Influence of temperature and moisture on the proportion hatching; all eggs laid in incubator "75 Wet."

(a) Incubator 75 Wet, moistened.

Date	Receptacle			Temperature	Moistened	Number examined	Number hatched	Percentage
	Tube	Box	Jar					
1 Dec. 1910	×			74.0	Water at start	11	11	100%
6 Dec. 1910	×			74.1	Water at start	21	18	85%
8 Dec. 1910	×			74.3	Water at start	14	14	100%
*28 Dec. 1910			×	74.6	Water at intervals	29	21	73%
13 Jan. 1911	×			74.7	Water at start	16	13	81%
* 4 Mar. 1911			×	74.6	Water 1 c.c. daily	36	13	36%
Total hatched	90%		53%					
*28 Dec. 1910			×	74.6	Urine at intervals	30	23	77%
* 4 Mar. 1911			×	74.6	Urine 1 c.c. daily	36	25	69%
Total hatched			73%					

(b) Incubator 75 Dry, moistened.

8 Dec. 1910	×			74.7	Water at start	19	12	63%
17 Dec. 1910	×			76.2	Water at start	25	21	84%
* 3 Jan. 1911			×	76.1	Water at intervals	21	13	62%
*31 Jan. 1911			×	76.6	Water daily	27	20	74%
28 Feb. 1911			×	76.0	Water 1 c.c. daily	30	17	57%
Total hatched	75%		64%					
* 3 Jan. 1911			×	76.1	Urine at intervals	19	12	63%
*31 Jan. 1911			×	76.6	Urine daily	39	21	54%
28 Feb. 1911			×	76.0	Urine 1 c.c. daily	30	15	50%
Total hatched			55%					

(c) Incubator 85 Wet, moistened.

1 Dec. 1910	×			83.5	Water at start	17	11	65%
8 Dec. 1910	×			83.8	Water at start	8	7	88%
*30 Dec. 1910			×	84.2	Water at intervals	20	16	80%
*24 Feb. 1911			×	83.7	Water 1 c.c. daily	36	21	58%
Total hatched	72%		66%					
*30 Dec. 1910			×	84.2	Urine at intervals	27	24	88%
*24 Feb. 1911			×	83.7	Urine 1 c.c. daily	36	19	53%
Total hatched			68%					

\* against experiments of the same date signifies that a single batch of eggs was divided.

## (d) Incubator 85 Dry, moistened.

Date	Receptacle			Temperature	Moistened	Number examined	Number hatched	Percentage
	Tube	Box	Jar					
*25 Nov. 1910			×	84.1	Water at intervals	15	8	53 0/0
1 Dec. 1910	×			83.8	Water at start	18	11	61 0/0
6 Dec. 1910	×			84.0	Water at start	20	18	90 0/0
8 Dec. 1910	×			84.0	Water at start	17	11	65 0/0
17 Dec. 1910	×			84.7	Water at start	24	19	79 0/0
*30 Dec. 1910			×	83.6	Water at intervals	17	15	88 0/0
*27 Jan. 1911			×	85.1	Water daily	16	9	56 0/0
Total hatched	75 0/0		66 0/0					
*25 Nov. 1910			×	84.1	Urine at intervals	18	13	88 0/0
*30 Dec. 1910			×	83.6	Urine at intervals	23	21	91 0/0
*27 Jan. 1911			×	85.1	Urine daily	10	4	40 0/0
Total hatched			75 0/0					

## (e) Warm Cupboard, moistened.

* 3 Feb. 1911			×	59.0	Water daily	25	19	76 0/0
* 8 Mar. 1911			×	60.0	Water 1 c.c. daily	35	16	48 0/0
Total hatched			58 0/0					
* 3 Feb. 1911			×	59.0	Urine daily	19	12	62 0/0
* 8 Mar. 1911			×	60.0	Urine 1 c.c. daily	35	12	34 0/0
Total hatched			42 0/0					

TABLE II. *Ova C. fasciatus*. Influence of temperature and humidity on proportion of eggs hatching; all eggs laid in incubator "75 Wet."

## (a) Incubator 75 Wet, unmoistened.

Date	Receptacle			Temperature	Humidity	Number examined	Number hatched	Percentage
	Tube	Box	Jar					
5 Aug. 1910	×			75	.66	5	4	80 0/0
11 Aug. 1910		×		75	.69	10	6	60 0/0
24 Sept. 1910			×	74	.73	20	16	80 0/0
30 Sept. 1910		×		74.1	.77	7	5	71 0/0
16 Oct. 1910	×			74	.73	15	15	100 0/0
19 Oct. 1910		×		74	.66	11	9	82 0/0
30 Oct. 1910	×			73.7	.72	{ 6 13	6 13	100 0/0 100 0/0
21 Dec. 1910			×	74.2	.75	32	28	87 0/0
13 Jan. 1911	×			74.7	.74	17	14	82 0/0
14 Feb. 1911	×			74.5	.76	14	10	71 0/0
14 Feb. 1911	×			74.5	.76	19	12	63 0/0
14 Feb. 1911	×			74.5	.76	18	12	67 0/0
Total hatched	80 0/0	72 0/0	85 0/0					

\* against experiments of the same date signifies that a single batch of eggs was divided.



(b) Cellar.

Date	Receptacle			Temperature		Humidity	Number examined	Number hatched	Percentage
	Tube	Box	Jar	Max.	Min.	Approximate			
31 July 1910	×			59	58	·93	2	1	50 %
4 Aug. 1910	×			59	58	·93	5	4	80 %
14 Aug. 1910	×			62	60	·93	3	1	33 %
7 Oct. 1910			×	58	57	·93	22	19	86 %
22 Oct. 1910	×			56	55	·93	7	3	43 %
Total hatched	53 %		86 %						

(c) Incubator 85 Dry, unmoistened.

24 Dec. 1910		×		83·8		·59	28	9	32 %
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(d) Incubator 85 Wet.

30 July 1910	×			85		·75	7	4	57 %
4 Aug. 1910	×			85		·74	6	4	66 %
10 Aug. 1910		×		85		·73	11	1	9 %
9 Sept. 1910		×		85		·70	5	3	60 %
22 Sept. 1910			×	85		·72	20	12	60 %
13 Oct. 1910	×			84·5		·72	11	9	82 %
5 Nov. 1910	×			84·7		·72	15	14	93 %
24 Dec. 1910			×	83·6		·70	26	16	62 %
Total hatched	74 %	25 %	61 %						

(e) Incubator 75 Dry.

21 Dec. 1910		×		75·7		·46	26	14	54 %
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(f) Warm Cupboard.

				Max.	Min.	About			
6 Aug. 1910	×			69	67	·60	3	3	100 %
10 Oct. 1910			×	74	69	·60	16	10	61 %
22 Oct. 1910	×			73	69	·60	6	5	83 %

(g) Laboratory Cupboard, unmoistened.

				Of Laboratory Approximate only		Approximate			
25 July 1910	×			60		·84	3	3	100 %
6 Aug. 1910		×		60		·84	5	2	40 %
12 Aug. 1910		×		60		·84	6	4	66 %
18 Aug. 1910	×			60		·84	6	3	50 %
27 Sept. 1910			×	60		·84	9	6	66 %
16 Oct. 1910		×		60		·84	16	15	94 %
19 Oct. 1910		×		60		·84	8	5	63 %
Total hatched	66 %	80 %	66 %						

TABLE III. *Ova C. fasciatus. Influence of varying the temperature and humidity upon the proportion of eggs of the same batch hatching; all eggs laid in incubator "75 Wet."*

Date 1910	Receptacle	Place	Unmoistened.		Humidity	No. exam.	No. hatched	Per- centage
19 Oct.	Box	Incubator 75 Wet	74		·66	11	9	82 0/0
		Lab. Cupboard	60 approx.		·84 approx.	8	7	87 0/0
22 Oct.	Tube		Max.	Min.				
		Warm Cupboard	73	69	·60	6	5	83 0/0
		Cellar	56	55	·90	7	3	43 0/0
Moistened.								
1 Dec.	Tube	Incubator 85 Wet	83·5		Water at start	17	11	65 0/0
		„ 85 Dry	83·8		„ „	18	11	61 0/0
6 Dec.	Tube	„ 75 Wet	74·1		„ „	21	18	85 0/0
		„ 85 Dry	84·0		„ „	20	18	90 0/0
8 Dec.	Tube	„ 75 Dry	74·7		„ „	19	12	63 0/0
		„ 85 Dry	84·0		„ „	17	11	65 0/0

NOTE. Moistened by means of a disk of blotting-paper beneath the sand with a projecting tag.

TABLE IV. Experiment with Ova C. fasciatus. The relative effect of varied conditions of Temperature and Humidity on (a) Fertility of eggs laid, (b) Hatching of eggs laid.

Place	Series I Females put in different places to lay and ova allowed to hatch in the same places as laid										Series II Females put into different places to lay. Ova hatched in Incubator 75 Wet				Series III Females all put into Incubator 75 Wet to lay. Ova distributed in batches into the different places to hatch			
	Number of females used	Date, 1911	Laid and hatched			No. of eggs laid	Percentage per female	Number hatched	Date, 1911	Hatched			Date, 1911	Temperature	Humidity	No. of eggs hatched		
			Temperature	Humidity	Number hatched					Temperature	Humidity	Percentage per female					Number hatched	
Incubator 75 Wet	50	11 April	75.3	.77	54	1.08	45 = 85%	7 July	76.7	.88	60	1.20	44 = 73%	18 July	76.3	.81	50	41 = 82%
	50	20 July	76.4	.82	89	1.78	63 = 71%											
Incubator 85 Wet	50	11 April	84.6	.71	65	1.30	43 = 66%	30 May	75.9	.90	78	1.56	54 = 69%	20 July	85.8	.83	50	33 = 66%
	50	20 July	85.8	.83	97	1.94	43 = 44%											
Incubator 75 Dry	50	20 April	75.5	.48	37	.74	26 = 70%	30 May	75.9	.90	41	.82	35 = 87%	19 July	76.6	.55	50	31 = 62%
	50	20 July	77.1	.56	51	1.01	38 = 75%											
Incubator 85 Dry	50	20 April	83.9	.64	82	1.64	42 = 51%	30 May	75.9	.90	42	.84	21 = 50%	20 July	84.1	.66	50	27 = 56%
	50	20 July	84.1	.66	85	1.70	36 = 42%											
Cellar	50	20 April	max. min. 51.7 50.1	.90	11	.22	3 = 27%											
Warm cupboard	50	24 July	65.6 64.4	.94	11	.22	9 = 82%	7 July	76.7	.88	58	1.16	46 = 79%	18 July	max. min. 62.9 61.8	.94	50	47 = 94%
	70	11 April	65.7	.53	23	.33	4 = 17%											
Laboratory cupboard	50	24 July	73.8	.62	3	.06	1 = 33%	7 July	76.7	.88	21	.42	14 = 67%	18 July	72.1	.62	50	28 = 56%
	60	11 April	max. min. 59.2 47.3	.76	8	.13	2 = 25%											
Beehive	50	24 July	74.2 65.7	.73	18	.36	15 = 83%	7 July	76.7	.88	43	.82	37 = 86%	18 July	max. min. 72.6 64.9	.72	50	31 = 62%
	50	20 April	67.0 34.6	—	20	.40	9 = 45%											
	50	25 July	82.1 69.2	—	6	.12	4 = 67%	7 July	76.7	.88	48	.96	37 = 77%	18 July	82.2 57.8	—	50	20 = 40%

TABLE V. *Ova P. irritans.* Influence of the temperature and humidity upon hatching of eggs; all eggs laid in incubator "75 Wet."

Incubator 75 Wet.									
Date	Tube	Box	Jar	Temperature	Humidity	No. examined	No. hatched	Percentage hatched	
1 Oct. 1910	×	—	—	74·1	·79	8	8	100 0/0	
10 „	—	—	×	74·2	·78	49	45	92 0/0	
10 „	×	—	—	74·2	·78	12	7	58 0/0	
*14 „	—	×	—	74·2	·75	23	15	65 0/0	
15 Feb. 1911	×	—	—	74·5	·76	23	17	74 0/0	
15 „	×	—	—	74·5	·76	23	12	52 0/0	
Incubator 85 Wet.									
26 Sept. 1910	—	—	×	85	·74	27	23	85 0/0	
14 Oct. 1910	—	×	—	84	·72	16	11	69 0/0	
Cellar.									
				Max.	Min.				
6 Oct. 1910	—	—	×	58	57	·90 approx.	21	9	43 0/0
*14 „	—	×	—	56	55	·90 „	18	11	61 0/0
Laboratory Cupboard.									
11 Aug. 1910	×	—	—	about 60	·83 approx.	12	9	75 0/0	
1 Oct. 1910	—	—	×	„ 60	·85 „	31	24	77 0/0	
Warm Cupboard.									
				Max.	Min.				
22 Sept. 1910	×	—	—	76	69	·60 approx.	8	3	37 0/0
18 Oct. 1910	—	×	—	73	69	·60 „	12	4	33 0/0

\* Signifies a portion of a divided batch of eggs.

TABLE VI. *Ova P. irritans*. Twenty females, fed for one week, were divided into four batches and kept in boxes with a number of males. These batches were then subjected to varied treatment as regards Temperature and Humidity.

Dates	Batch	Number of females	Number of males	Place where fleas were kept	Temperature	Humidity	Number of eggs laid per female per day	Number laid	Hatched	Place where eggs were kept till hatched
18 Aug.—1 Sept.	A	5	Av. 4.7	Incubator 75 Wet	75	.77	1.11	86	42=51%	Incubator 75 Wet
2—18 Sept.	A	Av. 4.4	6	" 85 Dry	84.3	.58	1.00	74	Nil	" 85 Dry
19 Sept.—9 Oct.	A	4	6	" 85 Dry	81.8	.58	1.21	102	36=35%	" 75 Wet
10 Oct.—1 Nov.	A	3	Av. 8.8	" 75 Wet	74.3	.74	1.55	107	53=49%	" 75 Wet
2 Nov.—25 Nov.	A	3	Av. 9.1	" 75 Wet	74.6	.70	1.18	85	35=41%	" 75 Wet
18 Aug.—1 Sept.	B	5	Av. 5	Incubator 75 Dry	74.6	.60	1.40	110	48=44%	Incubator 75 Dry
2—18 Sept.	B	5	6	Cellar	max. min. 62 61	.94	1.03	93	25=27%	Cellar
19 Sept.—9 Oct.	B	5	6	Cellar	57 56	.93	.43	45	9=20%	Incubator 75 Wet
10 Oct.—1 Nov.	B	5	Av. 9.1	Incubator 75 Dry	74.3	.53	1.61	185	108=58%	" 75 Wet
2 Nov.—25 Nov.	B	4.1	Av. 8	" 75 Wet	74.6	.70	1.71	171	87=50%	" 75 Wet
18 Aug.—1 Sept.	C	5	Av. 4.7	Incubator 85 Dry	83.9	.61	1.54	116	22=19%	Incubator 85 Dry
2—18 Sept.	C	Av. 4.2	Av. 5.6	" 75 Wet	75.1	.77	1.58	106	49=46%	" 75 Wet
19 Sept.—9 Oct.	C	4	6	" 75 Wet	74.2	.74	1.84	155	66=42%	" 75 Wet
10 Oct.—1 Nov.	C	Av. 3.8	Av. 9.1	" 85 Dry	84.5	.61	1.54	135	48=35%	" 75 Wet
2 Nov.—25 Nov.	C	3	Av. 9.1	" 75 Wet	74.6	.70	1.9	137	49=36%	" 75 Wet
18 Aug.—1 Sept.	D	5	Av. 5	Cellar	max. min. 63 62	.94	.70	53	25=47%	Cellar
2—18 Sept.	D	5	Av. 5.8	Incubator 75 Dry	75.1	.56	1.00	90	20=22%	Incubator 75 Dry
19 Sept.—9 Oct.	D	5	6	" 75 Dry	74.8	.51	1.19	125	55=44%	" 75 Wet
10 Oct.—1 Nov.	D	5	Av. 9.3	Cellar	max. min. 56 55	.93	.27	31	4=13%	" 75 Wet
2 Nov.—25 Nov.	D	Av. 4.6	Av. 9.6	Incubator 75 Wet	74.6	.70	.98	110	64=58%	" 75 Wet

TABLE VI (a). *Ova P. irritans*. Second portion of test. All batches of fleas A, B, C and D kept in Incubator 75 Wet. The ova laid by the several batches were "bulked," then divided into four lots and put under different conditions to hatch.

Place	Period of laying	Temperature	Humidity	Number of eggs	Number which hatched	Percentage which hatched	Aver.
Incubator 93 Dry	26 Nov.—13 Dec.	93.1	.56	81	9	11 0/10	
	14 Dec.—3 Jan.	92.9	.57	57	6	10 0/10	9 0/10
	4 Jan.—16 Jan.	93.0	.55	33	2	6 0/10	
Incubator 75 Dry	26 Nov.—13 Dec.	74.7	.49	81	Nil	—	
	14 Dec.—3 Jan.	74.8	.51	57	Nil	—	4 0/10
	4 Jan.—16 Jan.	74.4	.53	33	7	21 0/10*	
Incubator 75 Wet	26 Nov.—13 Dec.	76.0	.66	81	36	45 0/10	
	14 Dec.—3 Jan.	76.1	.66	57	35	61 0/10	52 0/10
	4 Jan.—16 Jan.	75.2	.74	33	18	54 0/10	
Cellar	26 Nov.—13 Dec.	max. 47.6 min. 45.8	.91	81	20	24 0/10	
	14 Dec.—3 Jan.	49.4 48.3	.93	57	18	32 0/10	26 0/10
	4 Jan.—16 Jan.	48.6 47.4	.93	33	6	18 0/10	

\* Owing to an alteration in Incubator the humidity rose to .66 for a period of 12 to 20 hours on the 5th Jan. and 4 eggs hatched out of a batch put in on the 3rd Jan. Of subsequent batches 3 eggs hatched with the humidity about .54 to .55 for the critical period; the last batch put in on a falling humidity failed to hatch.

TABLE VI (b). *Ova P. irritans. Egg Fertility test (compiled from Table VI). Females put under different conditions to lay eggs; all hatched in incubator 75 Wet.*

Batch	Place of laying	Conditions of laying		No. of eggs per female laid per day	Conditions of hatching		Percentage hatched
		Temperature °F.	Humidity		Temperature °F.	Humidity	
A	Incubator 75 Wet	74·3	·74	1·55	74·3	·74	49
B	„ 75 Dry	74·3	·53	1·61	74·3	·74	58
C	„ 85 Dry	84·5	·61	1·54	74·3	·74	35
D	Cellar	55·5	·93	·27	74·3	·74	13

TABLE VII. *Ova X. cheopis. Influence of temperature and humidity upon the hatching of eggs; all eggs laid in incubator 75 Wet.*

Date	Place of hatching	Temperature ° F.		Humidity	Number of eggs	Number hatched	Percentage
31 July 1911	Incubator 85 Wet	84·6		·81	25	15	60 %
1 Nov. 1911	„	84·6		·74	21	11	52 %
26 Nov. 1911	„	{ 3 days at 76		·65	24	11	46 %
		{ 4 „ 92·5		·67			
3 Jan. 1912	Incubator 93 Wet	92·6		·66	13	1	8 %
12 Oct. 1911	„ 85 Dry	85		·61	18	1	6 %
26 Nov. 1911	„ 93 Dry	93·4		·56	24	3	12 %
3 Jan. 1912	„ 93 Dry	93·2		·56	13	3	23 %
31 July 1911	Incubator 75 Wet	75·6		·84	25	19	76 %
12 Oct. 1911	„	74·7		·76	18	12	66 %
1 Nov. 1911	„	74·8		·70	24	14	58 %
3 Jan. 1912	„	75·5		·71	13	5	38 %
31 July 1911	Incubator 75 Dry	76		·60	25	12	48 %
12 Oct. 1911	„	75		·54	18	3	16 %
26 Nov. 1911	„	74·8		·49	24	Nil	—
3 Jan. 1912	„	75·2		·50	13	2	15 %
19 Oct. 1911	Warm Cupboard	70		·56	18	3	16 %
26 Nov. 1911	„	61		·58	24	Nil	—
31 July 1911	Cellar	Max.	Min.	·94	25	17	68 %
		64	63				
		57	55				
19 Oct. 1911	„	57	55	·93	18	Nil	—
26 Nov. 1911	„	48	46	·93	24	Nil	—
19 Oct. 1911	Beehive	60	42	—	18	Nil	—
26 Nov. 1911	„	45	34	—	24	Nil	—
26 Nov. 1911	Lab. Cupboard	53	45	·87	24	Nil	—

TABLE VIII. *Ova X. cheopis*. The relative effect of Temperature and Humidity on (a) Fertility of eggs laid, (b) Hatching of eggs laid.

Series I													Series II													Series III												
Females put in different places to lay and ova allowed to hatch in the same places as laid													Females put into different places to lay. Ova all put into incubator 75° Wet and allowed to hatch													Females all put into incubator 75° Wet to lay. Ova distributed in batches among the different test places												
Laid and hatched													Hatched													Hatched												
Place	Number of females used	Date, 1912	Temperature	Humidity	No. of eggs laid	Percentage per female	Number hatched						Date, 1912	Temperature	Humidity	No. of eggs laid	Percentage per female	Number hatched						Date, 1912	Temperature	Humidity	No. of eggs laid	Percentage per female	Number hatched									
Incubator 75° Wet	50	15 June	75.3	.83	15	.30	5 = 33%						29 July	74.2	.82	38	.76	15 = 39%						25 June	75.0	.86	10		5 = 50%									
	50	2 July	75.3	.80	12	.24	7 = 58%																9 Aug.	75.0	.81	18		15 = 83%										
	50	15 June	94.0	.81	17	.34	none						29 July	74.2	.82	23	.46	6 = 26%					25 June	95.9	.70	10		nil										
	50	2 July	95.4	.74	2	.04	none																9 Aug.	93.4	.78	18		5 = 27%										
Incubator 75° Dry	50	15 June	75.9	.61	12	.24	6 = 50%						29 July	74.2	.82	19	.38	4 = 21%					25 June	76.0	.59	10		3 = 30%										
	50	2 July	76.0	.59	16	.32	9 = 56%																9 Aug.	75.0	.57	18		6 = 33%										
Incubator 93° Dry	50	15 June	93.0	.57	17	.34	none						29 July	74.2	.82	30	.60	7 = 23%					25 June	93.0	.55	10		nil										
	50	2 July	93.0	.54	1	.02	none																9 Aug.	93.0	.54	18		nil										
Cellar	50	15 June	max. min. 57.6 56.5	.93	12	.24	3 = 25%						29 July	74.2	.82	1	.02	none					25 June	max. min. 58.0 57.2	.93	10		nil										
	50	2 July	58.5 57.7	.93	none	0	none																9 Aug.	57.5 56.5	.93	18		5 = 27%										
Warm Cupboard	50	15 June	66.5	.71	6	.12	1 = 16%						29 July	74.2	.82	21	.42	9 = 42%					25 June	66.7	.69	10		2 = 20%										
	50	2 July	64.8	.76	22	.44	5 = 22%																9 Aug.	64.6	.69	18		9 = 50%										
Laboratory Cupboard	50	2 July	max. min. 67.3 61.0	.81	5	.10	none						29 July	74.2	.82	3	.6	none					25 June	max. min. 64.3 59.6	.80	10		nil										
	50	15 June	70.4 52.3	—	8	.16	2 = 25%						29 July	74.2	.82	14	.28	2 = 14%					25 June	66.8 52.4	—	10		nil										
Beehive	20	15 June	41.9	.98	none	0	none						25 June	41.7	.95	10		4 = 22%					25 June	41.7	.95	10		nil										
	20	15 June	41.9	.98	none	0	none						25 June	41.7	.95	10		4 = 22%					25 June	41.7	.95	10		nil										



TABLE IX. *Ova Ct. canis.* A number of fleas found in the dog's bed. These were placed in a box and put into Incubator 75 Wet. The eggs were divided and batches put into the different Incubators and Cupboards to hatch.

Date	Place	Temperature	Humidity	Number of eggs	Number hatched	Percentage hatched
26 July 1911	Incubator 85 Wet	85·3	·82	10	7	70 %
„	„ 75 Wet	76·2	·83	10	8	80 %
„	„ 75 Dry	76·3	·60	10	5	50 %
		Max. Min.				
„	Cellar	64·5 63·4	·94	10	8	80 %
„	Lab. Cupboard	73·0 65·0	·74	10	7	70 %
18 Sept. 1911	Incubator 75 Wet	74·6	·76	17	11	64 %

## 2. LARVAE.

(i) *Ability of newly hatched larvae to exist without food in case.* In Buckland's *Curiosities of Natural History* (Appendix to Fourth Series) a paper on "Fleas," read by the Rev. J. Hussey before the Ashmolean Society of Oxford in 1836, is given as the authority for the statement that young larvae can exist for from two to three weeks without food.

The following experiments have been carried out to test this point:

**METHOD.** A number of newly hatched larvae were put away in small tubes with three or four scraps of blotting-paper to afford foothold and cover. Coloured blotting-paper is to be preferred, as it shows up the larvae and renders counting easier. In most of the experiments the tubes were corked, but, in order to test if this made any serious difference to the results, a few tests were made with cotton-wool plugs in place of corks and sand in place of the blotting-paper. Any dead larvae were removed daily to avoid all possibility of the survivors feeding on them. (See Tables X, XI and XII.)

While the results obtained support the correctness of Hussey's statement, the differing conditions of temperature and humidity were also found to have a very marked influence. So different were the results with *C. fasciatus* in mid-winter and *P. irritans* in the spring, that a number of repetitions of the experiment were made in order to test how far the difference was specific and how far a question of season. It appears that *C. fasciatus* is much better adapted to survive unfed than *P. irritans*; in one instance, in the cellar, in the winter the average length of life of a batch of larvae without food was 75 days and some individuals survived over 100 days. (See Table X.) There is an apparent well-marked seasonal change in the powers of endurance of *C. fasciatus*, as shown by a comparison of the average length of life

in the incubators during winter and summer. This apparent lack of endurance of summer hatched larvae is really more marked than at first sight appears, if we consider the fact that the humidity in the incubators is higher in the summer than in the winter<sup>1</sup>. Judging, however, by the fact that this seasonable difference is confined to the experiments with incubators and is not traceable in the cellar or warm cupboard records, *it must be concluded that this wide discrepancy is due to the difference in the ventilation of the incubators in summer and winter*. Throughout the whole course of these experiments the ventilators have been open as freely as possible consistent with the required percentage of humidity. This results in keeping the ventilators of the Wet incubators all but shut during the winter.

There is some evidence of seasonal difference in the powers of endurance of *P. irritans* to be gleaned from Table XI. The differences between the March and June series of *P. irritans* are probably due to individual variation and the small numbers used in the March experiments. The results of the later tests with larger numbers only show the influence of favourable or unfavourable conditions of humidity and temperature.

As will be seen from the tables, *X. cheopis* comes between *C. fasciatus* and *P. irritans* as regards its powers of endurance unfed. Tubes with cotton-wool plug and a little sand at the bottom gave a better result than the corked tubes with blotting-paper under moist and moderately cool conditions. With regard to the experiment in incubators 75 Dry on the 15th July, 1912, I am inclined to question the humidity record; the readings for a few days about this period were very irregular and quite abnormal and it is possible that the high records may have been merely snatch readings. If the humidity really averaged .71, and did not sink below .63, it is remarkable that the larvae lived so short a time.

Although *X. cheopis* is normally an inhabitant of hot climates, it is surprising that the newly-hatched larvae of this species are not able to survive hot and dry conditions any better than *C. fasciatus* (see Table XII).

A small trial with *Ct. canis* in July, 1911 (see Table XIII), suggests that this species is scarcely so well adapted to exist unfed as *P. irritans*; it is certainly very inferior in this respect to *C. fasciatus*.

<sup>1</sup> Newly hatched larvae in small tubes might possibly be utilised as a method of testing the conditions of humidity prevailing in corners, holes in walls, etc. where it is not possible to take readings with an hygrometer.

Advantage is apparently taken by *C. fasciatus*, and possibly by *P. irritans* as well, of these powers of prolonged fasting to increase the variability of the brood in the matter of emergence. In some rearing experiments, it was noticed that the larvae when given food did not all commence to grow for varying periods of from several days to two or three weeks and a few larvae became no larger than when they emerged from the egg. Meanwhile their fellows of the same batch had attained their full growth.

To test the crawling powers of the freshly emerged unfed larvae, the tracks of several individuals of *C. fasciatus*, *P. irritans* (Fig. 2, p. 502) and *X. cheopis* (Fig. 3, p. 503) were followed with a pencil on sheets of paper, the tests lasting for 30 minutes in each case.

It will be noted that both *P. irritans* and *X. cheopis* seem better adapted for wandering in search of food than *C. fasciatus*. An attempt was also made with *C. fasciatus* and *X. cheopis* to ascertain if the freshly emerged larvae crawled quite at random or whether they showed any instinctive tendency to progress in the direction of food, moisture or cover.

Eleven newly hatched larvae of *C. fasciatus* were placed on a large sheet of white paper with the following substances arranged in a circle at equal distances from their starting point: (a) a piece of blood-soaked rag, (b) a piece of gauze spotted with flea faeces, (c) a piece of plain cloth, and (d) a pat of moist sand. The larvae crawled in various directions, but none went near the cloth; two approached quite close to the damp sand, but turned aside and wandered past it. Three came quite close to the gauze with the flea droppings on it, but seemed quite uninfluenced by the proximity of this food, and either wandered past it or turned back towards their starting point. None went anywhere near the blood-soaked rag. The only conclusion possible during the period of watching was that their wanderings were quite at random and in no way influenced by the various articles.

The experiment was repeated with *X. cheopis*, the only difference being that the flea-spotted gauze was replaced by dry sand. Fifteen newly hatched larvae were placed in a central circle and watched for 15 minutes, with the following result:

- 5 did not leave the circle.
- 1 reached the wet sand.
- 1 reached the dry sand.
- 1 reached the cloth.

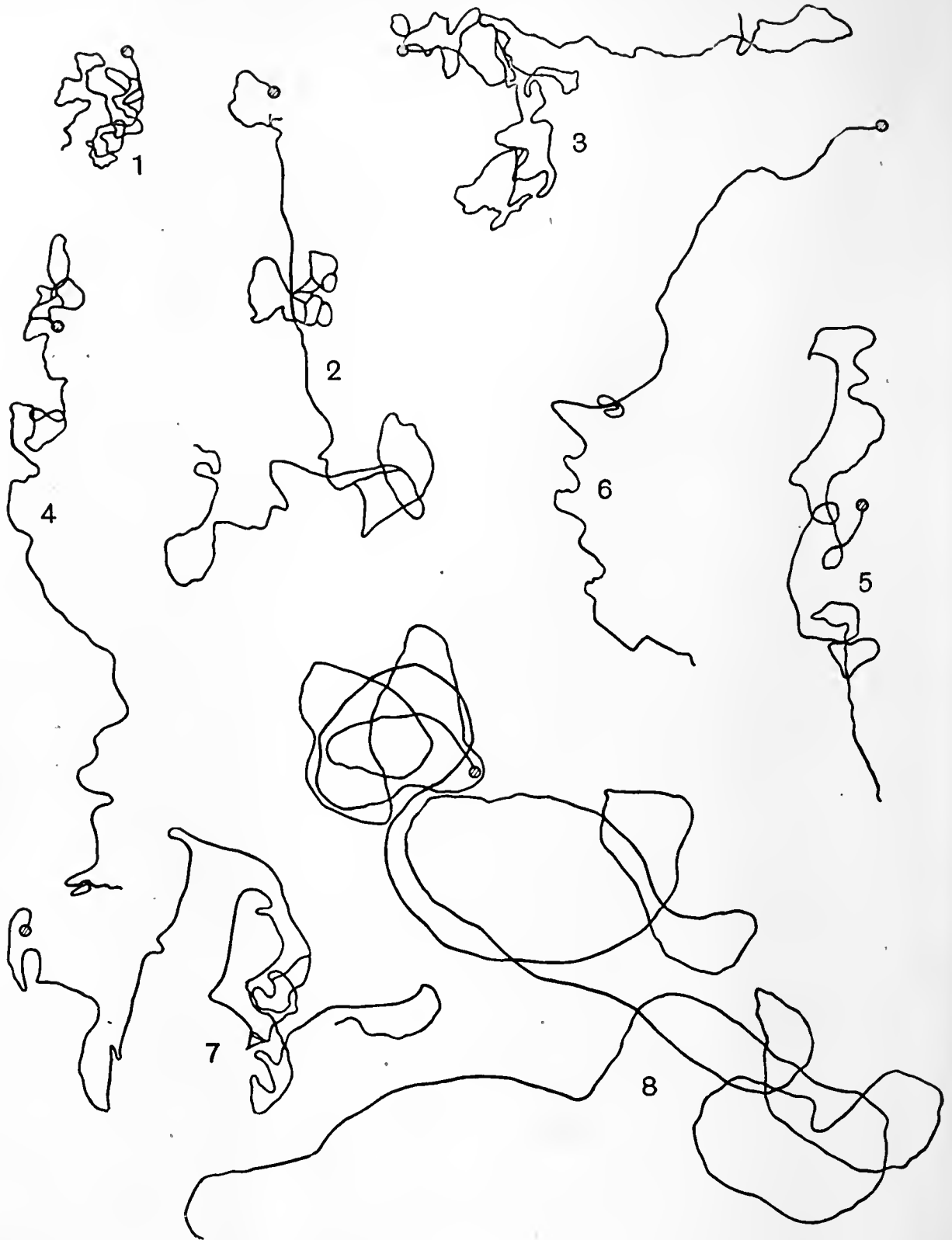


Fig. 2. 1. Larva of *C. fasciatus*. Newly hatched. 30 minutes on white paper. Distance=about 6 inches. 2. Larva of *C. fasciatus*. Newly hatched. 30 minutes on white paper. Distance=about 15 inches. 3. Larva of *C. fasciatus*. Newly hatched. 30 minutes on white paper. Distance=about 16 inches. 4. Larva of *C. fasciatus*. Newly hatched. 30 minutes on white paper. Distance=about 12 inches. 5. Larva of *C. fasciatus*. Newly hatched. On cork carpet 30 minutes. Distance=about 11 inches. 6. Larva of *C. fasciatus*. Newly hatched. On cork carpet 30 minutes. Distance=about 8 inches. 7. Larva *P. irritans*. Newly hatched. On cork carpet 30 minutes. Distance=about 18 inches. 8. Larva of *P. irritans*. Newly hatched. On white paper 30 minutes. Distance=about 42 inches.



Fig. 3. 9. No. 1 Larva of *X. cheopis*. Newly hatched. On white paper 30 minutes. Distance=about 16 inches. 10. No. 2 Larva of *X. cheopis*. Newly hatched. On white paper 30 minutes. Distance=about 27 inches. 11. No. 3 Larva of *X. cheopis*. Newly hatched. On white paper 30 minutes. Distance=about 23 inches. 12. No. 4 Larva of *X. cheopis*. Newly hatched. On white paper 30 minutes. Distance=about 23 inches.

- 1 stopped to feed just before reaching the blood-soaked rag, presumably on a speck of dried blood which had become detached, as its alimentary canal at once darkened.
- 1 wandered out past and between the wet and dry sand.
- 3 remained sluggish after crawling about midway between the starting circle and the wet sand.
- 1 stopped between the starting circle, blood-soaked rag and the wet sand.

The larvae are, however, very prompt at attacking food in their immediate proximity. A larva has been seen to partake of a speck of flea faeces when placed in the lid of a small cardboard box that was apparently quite clean, but was found to have a few specks of flea droppings on it when carefully examined. It was a matter of a few seconds only for the perfectly white freshly emerged larvae to show a dark line as the food entered its stomach.

TABLE X. *Newly hatched larvae C. fasciatus. Experiment to determine length of life unfed. Series I.*

A. In corked tubes.

Date	Place	Temperature max. min.	Average Humidity	Number put into tubes	Detail	Average life
13 Dec. '10	Cellar	49 48	·93	8	2 dead in 9 days	23 days
					1 „ 19 „	
					1 „ 25 „	
					2 „ 29 „	
					2 „ 31 „	
14 Dec. '10	Incubator 75 Wet	74·4	·75	7	2 „ 13 „	18 „
					2 „ 18 „	
					1 „ 20 „	
					2 „ 24 „	
17 Dec. '10	Incubator 85 Wet	84·4	·69	8	2 „ 10 „	14 „
					6 „ 15 „	
18 Dec. '10	Lab. Cupboard	max. min. 53 44	·84	10	1 „ 10 „	26 „
					2 „ 16 „	
					2 „ 26 „	
					2 „ 29 „	
					1 „ 33 „	
					2 „ 39 „	
18 Dec. '10	Incubator 85 Dry	85	·59	10	4 „ 3 „	5 „
					4 „ 5 „	
					2 „ 8 „	
19 Dec. '10	Incubator 75 Dry	76	·43	10	all „ 3 „	3 „
19 Dec. '10	Warm Cupboard	64	·57	10	„ „ 3 „	3 „
19 Dec. '10	Beehive	*max. min. 44 32		10	2 „ 13 „	23 „
					7 „ 23 „	
					1 „ 39 „	
22 July '11	Warm Cupboard	74	·62	12	5 „ 2 „	4 „
					3 „ 4 „	
					1 „ 5 „	
					3 „ 7 „	
22 July '11	Beehive	max. min. 85·5 59·5	—	12	11 „ 2 „	2·4 „
					1 „ 7 „	
23 July '11	Lab. Cupboard	73·3 65·3	·73	12	6 „ 2 „	6 „
					1 „ 3 „	
					1 „ 5 „	
					2 „ 10 „	
					1 „ 13 „	
					1 „ 18 „	
23 July '11	Cellar	64·5 63·5	·94	12	1 „ 2 „	26·2 „
					4 „ 19 „	
					2 „ 24 „	
					2 „ 36 „	
					3 „ 39 „	

\* Average temperature for the last 29 days only.

TABLE X.—*continued.*

Date	Place	Temperature	Average Humidity	Number put into tubes	Detail	Average life
23 July '11	Incubator 75 Wet	75·9	·82	12	2 dead in 3 days	10·8 days
					1 ,, 4 ,,	
					2 ,, 5 ,,	
					3 ,, 12 ,,	
					1 ,, 16 ,,	
					2 ,, 18 ,,	
					1 ,, 22 ,,	
23 July '11	Incubator 75 Dry	77·6	·52	12	9 ,, 1 day	1·3 ,,
					3 ,, 2 days	
23 July '11	Incubator 85 Wet	86	·82	12	5 ,, 1 day	3·3 ,,
					1 ,, 2 days	
					2 ,, 3 ,,	
					1 ,, 4 ,,	
					2 ,, 8 ,,	
					1 ,, 11 ,,	
23 July '11	Incubator 85 Dry	84	·68	12	10 ,, 1 day	1·4 ,,
					1 ,, 3 days	
					1 ,, 4 ,,	
29 Nov. '11	Incubator 75 Wet	75·8	·65	12	1 ,, 6 ,,	20 ,,
					1 ,, 13 ,,	
					3 ,, 20 ,,	
					2 ,, 23 ,,	
					3 ,, 24 ,,	
					1 ,, 25 ,,	
					1 ,, 26 ,,	
29 Nov. '11	Incubator 75 Dry	75·2	·49	12	all ,, 2 ,,	
29 Nov. '11	Incubator 92 Wet	92·5	·67	12	1 ,, 1 day	5 ,,
					1 ,, 3 days	
					1 ,, 4 ,,	
					1 ,, 5 ,,	
					5 ,, 6 ,,	
					3 ,, 7 ,,	
29 Nov. '11	Incubator 92 Dry	93·8	·57	12	3 ,, 1 day	3 ,,
					3 ,, 2 days	
					1 ,, 3 ,,	
					4 ,, 4 ,,	
					1 ,, 7 ,,	
11 Dec. '11	Cellar	max. min. Dec. 48·7 47·4 Jan. 47·2 45·9 Feb. 45·8 44·6	·92 ·92 ·92	9	3 ,, 57 ,, 2 ,, 64 ,, 2 ,, 77 ,, 1 ,, 101 ,, 1 ,, 120 ,,	75 ,,
24 Dec. '11	Lab. Cupboard	Dec. 53·9 46·2 Jan. 52·5 44·9 Feb. 54·4 45·3	·87 ·89 ·86	10	2 ,, 5 ,, 1 ,, 11 ,, 1 ,, 32 ,, 3 ,, 43 ,, 2 ,, 51 ,, 1 ,, 53 ,,	34 ,,



TABLE X.—*continued.*

Date	Place	Temperature	Average Humidity	Number put into tubes	Detail		Average life
24 Dec. '11	Warm Cupboard	60·4	·62	9	4 dead in	4 days	5 days
					4 „	6 „	
					1 „	7 „	
25 Dec. '11	Beehive	Dec. <sup>max.</sup> 46·8 <sup>min.</sup> 35·0	—	12	2 „	5 „	50 „
		Jan. 43·6 34·0			2 „	10 „	
		Feb. 46·7 34·3			4 „	77 „	
					2 „	87 „	
					1 „	89 „	
25 Dec. '11	Incubator 92 Wet	92	·71	13	13 „	1 day	
26 Dec. '11	Incubator 92 Wet	92·5	·71	3	3 „	1 „	
<b>B. Placed in small glass tubes containing a little sand and with cotton-wool plugs in place of corks.</b>							
18 Mar. '12	Incubator 75 Wet	75·2	·80	10	2 „	11 days	17 „
					2 „	14 „	
					2 „	17 „	
					3 „	21 „	
					1 „	22 „	
18 Mar. '12	Incubator 75 Dry	72	·54	10	9 „	1 day	1 day
					1 „	2 days	
18 Mar. '12	Incubator 92 Wet	91·5	·68	10	3 „	4 „	6 days
					7 „	7 „	
18 Mar. '12	Incubator 92 Dry	92	·59	9	6 „	1 day	1 day
					2 „	2 days	
					1 „	3 „	
18 Mar. '12	Cellar	Mar. <sup>max.</sup> 49·1 <sup>min.</sup> 48·1	·93	9	2 „	17 „	34 days
		Apr. 50·3 49·1	·93		2 „	22 „	
		May 54·2 53·2	·93		2 „	29 „	
					1 „	39 „	
					1 „	59 „	
					1 „	78 „	
18 Mar. '12	Beehive	Mar. 54·1 38·2	—	10	2 „	7 „	29 „
		Apr. 64·1 37·3			1 „	11 „	
					1 „	14 „	
					2 „	17 „	
					1 „	22 „	
					1 „	26 „	
					1 „	33 „	
					1 „	35 „	
Mar. '12	Warm Cupboard	57·5	·63	10	all „	2 „	
18 Mar. '12	Lab. Cupboard	<sup>max.</sup> 54·4 <sup>min.</sup> 45·5	·86	10	3 „	3 „	7 „
					3 „	7 „	
					3 „	10 „	
					1 „	12 „	

TABLE XI. *Newly hatched larvae P. irritans. Experiment to determine length of life unfed.*

## A. In corked tubes.

Date	Place	Temperature	Average Humidity	Number put into tubes	Detail	Average life
15 Mar. '11	Incubator 85 Dry	84	.55	1	1 dead in 2 days	2 days
14 Mar. '11	Incubator 75 Dry	75	.48	3	1 ,, 1 day	3 ,,
					1 ,, 3 days	
					1 ,, 5 ,,	
16 Mar. '11	Incubator 85 Wet	83	.70	3	3 ,, 1 day	1 day
17 Mar. '11	Incubator 85 Wet	83	.70	1	1 ,, 1 ,,	
13 Mar. '11	Incubator 75 Wet	74	.76	6	4 ,, 5 days	6 days
					2 ,, 7 ,,	
18 Mar. '11	Lab. Cupboard	max. min. 56 47	.86	2	1 ,, 12 ,,	13 ,,
					1 ,, 15 ,,	
19 Mar. '11	Beehive	55 34	—	4	1 ,, 4 ,,	19 ,,
					2 ,, 24 ,,	
					1 ,, 26 ,,	
20 Mar. '11	Cellar	46 44	.92	4	2 ,, 15 ,,	18 ,,
					1 ,, 19 ,,	
					1 ,, 23 ,,	
21 Mar. '11	Warm Cupboard	61	.61	2	1 ,, 1 day	5 ,,
					1 ,, 9 days	
24 Mar. '11	Incubator 75 Wet	75	.74	4	4 ,, 6 ,,	
3 April '11	Incubator 75 Wet	75	.76	8	2 ,, 4 ,,	6 ,,
					5 ,, 7 ,,	
					1 ,, 10 ,,	
3 April '11	Incubator 85 Wet	84	.68	6	1 ,, 1 day	4 ,,
					1 ,, 3 days	
					2 ,, 4 ,,	
					1 ,, 5 ,,	
					1 ,, 6 ,,	
*21 June '11	Cellar	max. min. 58 57	.93	12	6 ,, 9 ,,	12 ,,
					5 ,, 14 ,,	
					1 ,, 19 ,,	
*21 June '11	Lab. Cupboard	63 59	.85	12	2 ,, 5 ,,	9 ,,
					9 ,, 9 ,,	
					1 ,, 12 ,,	
†22 June '11	Incubator 85 Dry	83	.68	12	4 ,, 2 ,,	3 ,,
					8 ,, 3 ,,	
†22 June '11	Incubator 85 Wet	84	.79	12	5 ,, 4 ,,	5 ,,
					5 ,, 5 ,,	
					2 ,, 6 ,,	
‡26 June '11	Incubator 75 Wet	75	.74	12	3 ,, 3 ,,	4 ,,
					8 ,, 4 ,,	
					1 ,, 5 ,,	
‡26 June '11	Incubator 75 Dry	75	.55	12	12 ,, 1 day	1 day

\* Batches of larvae were divided on each date \* † ‡.

TABLE XI.—*continued.*

Date	Place	Temperature	Average Humidity	Number put into tubes	Detail	Average life
20 July '11	Incubator 75 Wet	76·5	·82	12	3 dead in 4 days	6 days
					8 " 6 "	
					1 " 8 "	
20 July '11	Incubator 75 Dry	77	·56	12	5 " 2 "	3 "
					4 " 3 "	
					2 " 4 "	
					1 " 5 "	
20 July '11	Incubator 85 Wet	85·8	·83	12	3 " 3 "	4 "
					4 " 4 "	
					5 " 5 "	
20 July '11	Incubator 85 Dry	84	·72	12	4 " 2 "	3 "
					8 " 3 "	
22 July '11	Cellar	max. min. 64 63	·94	12	3 " 3 "	6 "
					2 " 4 "	
					6 " 6 "	
					1 " 14 "	
22 July '11	Lab. Cupboard	75·2 66·7	·70	12	8 " 3 "	4 "
					3 " 6 "	
					1 " 7 "	
22 July '11	Warm Cupboard	75	·60	12	8 " 2 "	2 "
					2 " 3 "	
					2 " 4 "	
22 July '11	Beehive	max. min. 86 59	—	12	1 " 2 "	4 "
					8 " 4 "	
					2 " 5 "	
					1 " 6 "	
29 Nov. '11	Incubator 75 Wet	76·5	·67	11	2 " 2 "	5 "
					4 " 5 "	
					2 " 6 "	
					2 " 7 "	
					1 " 8 "	
29 Nov. '11	Incubator 75 Dry	75·2	·49	11	all " 2 "	
29 Nov. '11	Incubator 92 Wet	92	·66	11	" " 1 day	
29 Nov. '11	Incubator 92 Dry	93·7	·57	11	9 " 1 "	1 day
					2 " 2 days	
2 Dec. '11	Warm Cupboard	64·6	·59	12	1 " 2 "	3 days
					7 " 3 "	
					4 " 4 "	
11 Dec. '11	Cellar	max. min. 48·8 47·7	·93	12	4 " 11 "	14 "
					2 " 14 "	
					2 " 15 "	
					2 " 16 "	
					1 " 18 "	
					1 " 20 "	

TABLE XI.—*continued.*

Date	Place	Temperature max. min.	Average Humidity	Number put into tubes	Detail	Average life
11 Dec. '11	Lab. Cupboard	54.4 47.4	.87	12	3 dead in 8 days	10 days
					9 ,, 11 ,,	
12 Dec. '11	Beehive	47.4 35.2	—	12	7 ,, 4 ,,	7 ,,
					2 ,, 7 ,,	
					2 ,, 10 ,,	
					1 ,, 23 ,,	
<b>B. In small glass tubes plugged with cotton-wool and a little sand at bottom.</b>						
1 Jan. '12	Incubator 75 Wet	76.8	.65	10	3 ,, 2 ,,	4 ,,
					2 ,, 3 ,,	
					5 ,, 5 ,,	
1 Jan. '12	Incubator 92 Wet	93.5	.68	10	3 ,, 1 day	2 ,,
					3 ,, 2 days	
					4 ,, 3 ,,	
1 Jan. '12	Incubator 92 Dry	94	.54	10	6 ,, 1 day	1 day
					4 ,, 2 days	
1 Jan. '12	Incubator 75 Dry	75.5	.49	10	4 ,, 2 ,,	3 days
					4 ,, 3 ,,	
					2 ,, 4 ,,	
20 Jan. '12	Cellar	max. min. 46.2 44.5	.92	10	3 ,, 5 ,,	9 ,,
					5 ,, 10 ,,	
					2 ,, 14 ,,	
20 Jan. '12	Beehive	43.2 35.1	—	10	7 ,, 5 ,,	6 ,,
					2 ,, 7 ,,	
					1 ,, 9 ,,	
22 Jan. '12	Warm Cupboard	58.5	.63	10	8 ,, 2 ,,	2 ,,
					2 ,, 3 ,,	
22 Jan. '12	Lab. Cupboard	max. min. 51.1 42.8	.90	10	4 ,, 4 ,,	7 ,,
					3 ,, 8 ,,	
					3 ,, 12 ,,	
25 Jan. '12	Incubator 75 Dry	75	.52	10	8 ,, 1 day	1 day
					2 ,, 2 days	
26 Jan. '12	Incubator 75 Wet	75.6	.71	10	6 ,, 5 ,,	6 days
					3 ,, 8 ,,	
					1 ,, 10 ,,	
29 Jan. '12	Incubator 93 Wet	93	.65	10	4 ,, 2 ,,	3 ,,
					6 ,, 3 ,,	
29 Jan. '12	Incubator 93 Dry	94	.56	10	2 ,, 1 day	2 ,,
					7 ,, 2 days	
					1 ,, 3 ,,	
30 Jan. '12	Beehive	max. min. 40 27	—	10	all ,, 4 ,,	
	Daily readings	41 26				
		36 19				
		42 12				
30 Jan. '12	Cellar	40.5 39.4	.91	10	7 ,, 7 ,,	9 ,,
					2 ,, 14 ,,	
					1 ,, 16 ,,	

TABLE XII. *Newly hatched larvae X. cheopis. Experiment to determine length of life unfed.*

**A. In corked tubes.**

Date	Place	Temperature max. min.		Average Humidity	Number put in tubes	Detail	Average life
14 June '11	Cellar	57	56	·93	6	2 dead in 12 days	16 days
						2 " 16 "	
						2 " 19 "	
4 Sept. '11	Cellar	60	58	·93	12	2 " 10 "	19 "
						3 " 14 "	
						1 " 16 "	
						2 " 18 "	
						3 " 28 "	
						1 " 30 "	
4 Sept. '11	Lab. Cupboard	67	58	·75	12	3 " 8 "	10 "
						4 " 10 "	
						2 " 11 "	
						1 " 12 "	
						2 " 15 "	
4 Sept. '11	Incubator 75 Wet	74·8		·76	12	3 " 7 "	11 "
						4 " 10 "	
						2 " 12 "	
						1 " 14 "	
						1 " 15 "	
						1 " 26 "	
4 Sept. '11	Incubator 85 Wet	84·9		·79	12	4 " 4 "	6 "
						6 " 6 "	
						2 " 8 "	
28 Sept. '11	Incubator 85 Dry	84·9		·57	12	4 " 4 "	5 "
						7 " 5 "	
						1 " 6 "	
28 Sept. '11	Incubator 75 Dry	75		·50	12	9 " 2 "	2 "
						3 " 3 "	
28 Sept. '11	Warm Cupboard	67		·64	12	12 " 3 "	3 "
11 Dec. '11	Incubator 75 Wet	75·6		·67	7	4 " 11 "	12 "
						1 " 13 "	
						1 " 14 "	
						1 " 15 "	
11 Dec. '11	Incubator 92 Wet	92·1		·67	7	2 " 2 "	4 "
						1 " 4 "	
						2 " 5 "	
						2 " 6 "	
24 Dec. '11	Incubator 75 Dry	74·7		·51	9	1 " 2 "	5 "
						4 " 4 "	
						3 " 6 "	
						1 " 7 "	
13 July '12	Incubator 93 Wet	95·2		·83	8	5 " 2 "	3 "
						2 " 3 "	
						1 " 5 "	
15 July '12	Incubator 93 Dry	93		·69	6	5 " 1 day	1 day
						1 " 2 days	

TABLE XII.—continued.

Date	Place	Temperature	Average Humidity	Number put in tubes	Detail	Average life
15 July '12	Incubator 75 Dry	77.5	.71*	6	3 dead in 1 day 3 " 2 days	1 day
<b>B. Placed in small glass tubes with a little fine sand; the tubes plugged with cotton-wool.</b>						
4 Aug. '12	Incubator 75 Wet	74.7	.81	6	3 " 13 " 3 " 22 "	17 days
4 Aug. '12	Incubator 75 Dry	75	.64	6	5 " 1 day 1 " 2 days	1 day
4 Aug. '12	Incubator 93 Wet	93.3	.78	6	2 " 1 day 1 " 4 days 1 " 6 " 2 " 9 "	5 days
4 Aug. '12	Incubator 93 Dry	93	.54	6	6 " 1 day	1 day or less
5 Aug. '12	Warm Cupboard	65.1	.72	4	1 " 2 days 1 " 4 " 2 " 6 "	4 days
5 Aug. '12	Cellar	Aug. Sept. max. min. 57.5 56.5	.93	6	2 " 10 " 1 " 12 " 1 " 29 " 2 " 35 "	22 "
15 Aug. '12	Lab. Cupboard	62.4 54.5	.86	6	1 " 5 " 1 " 15 " 1 " 20 " 1 " 25 " 1 " 28 " 1 " 30 "	20 "
15 Aug. '12	Beehive	64.5 44.7	—	6	1 " 4 " 1 " 19 " 1 " 22 " 1 " 25 " 2 " 28 "	21 "

\* Humidity very irregular; varied from .79 to .63.

TABLE XIII. *Newly hatched larvae Ct. canis. Experiment to determine length of life unfed.*  
In corked tubes.

Date	Place	Temperature	Average Humidity	Number put in tubes	Detail	Average life
29 July '11	Incubator 75 Wet	76	.85	10	6 dead in 2 days 2 " 4 " 1 " 6 " 1 " 10 "	4 days
29 July '11	Incubator 75 Dry	77.7	.59	10	7 " 2 " 3 " 4 "	3 "
29 July '11	Cellar	max. min. 63 62	.94	10	4 " 3 " 5 " 7 " 1 " 13 "	6 "

(ii) *Influence of temperature, humidity and nature of the food supply upon rearing.* The chief factors influencing the larval life of the species experimented on appear to be humidity of the air, temperature, and food supply. A situation sufficiently dry to ensure that dust and fine sand do not cling is essential for rearing larvae, while at the same time the percentage of humidity in the air in their immediate proximity should be above a mean of '60 to '65. It is of course probable that owing to local moistening larvae may thrive amid what are otherwise impossible circumstances. For example, the margins of a periodically wetted area in a very dry place would give limited but quite feasible opportunities for larval life; being very active crawlers, the larvae could easily keep to the margins, even if the area fluctuated in size. The numerous instances of larvae quitting their cocoons shortly after spinning, when the latter were subjected to hot, dry conditions, may indicate an instinct on the part of the larva, if an unfavourable change occurs, to search for a more suitable situation for its metamorphosis, even after the cocoon has been formed.

While it is difficult to fix exact limits to the range of temperature to which larvae can adjust themselves, it is certain that unfavourable, but not necessarily fatal conditions, may enormously delay development. Cold delays development in some species, and is fatal to others.

As regards food, the range of possible diet is no doubt a wide one, as already noted in the Committee's Report (Vol. VIII, No. 2, May 1908, *Journal of Hygiene*), but some of my experiments have shown that all the species are not alike in this respect and that some do not succeed on food that gives good results for others.

*P. irritans* is not so particular about its food as *C. fasciatus*; it can also survive in draughty situations or under conditions of humidity that are fatal to *C. fasciatus*.

*X. cheopis* appears to resemble *P. irritans*, rather than its congener, in the matter of food, but is more susceptible to cool conditions than the other species dealt with, and possibly better able to resist dry heat. This difference in adaptability on the part of *X. cheopis* and *C. fasciatus* in respect to food has probably a direct relation to the habits of their hosts, assuming, as I do, that the Committee's Report ("On the Bionomics of Fleas," Vol. VIII, No. 2, p. 245, May 1908, *Journal of Hygiene*) is correct in considering *M. rattus* as the host of *X. cheopis* and *M. decumanus* as the host of *C. fasciatus*. *M. rattus* is apparently chiefly to be found in houses, warehouses, granaries, etc., places which would be likely to offer many situations in which larvae might be reared quite apart from their hosts, provided that they could make

shift on a diet devoid of their parents' droppings. On the other hand, *M. decumanus*, as an inhabitant of sewers and cellars, is more associated with the ground level or beneath it and occupies situations which are less likely to afford suitable places for the rearing of larvae apart from the nests of their hosts. The habits of the two fleas fit those of their respective hosts. Compared with *C. fasciatus*, *X. cheopis* is far more closely attached to the rat; accustomed to travel with its host, it can afford to drop some of its eggs with a fair chance of the resulting larvae being reared. Although, under these circumstances, the droppings of the adult fleas are likely to be thinly scattered over a wide area, this need not prove disastrous for rearing the larvae of this species which are not entirely dependent upon food in this form. Supposing that the adults of *C. fasciatus* had the same habit of clinging to the rat that *X. cheopis* has, such a habit would be unduly wasteful, as numbers of ova might fall in places where there would be no chance of a food supply other than the thinly scattered flea droppings.

The cool, and probably more humid, situations in which *M. decumanus* must frequently nest, would make it advantageous for the adult fleas to wait quite a considerable time for the return of their host to the nest, for among the haunts of the animal it is likely to be one of the few favourable situations for flea breeding.

*C. fasciatus*. The experiments (see Tables XV to XVIII) show that a cool situation with a humid atmosphere is most favourable to the larvae of this species, but that temperature up to 85° F. may be survived by some individuals; low humidities at any temperature will prove fatal to active larvae. Under the conditions of the tests the critical point of humidity was found to be in the neighbourhood of .60 to .65 for temperatures up to 75° F. in a still atmosphere. With a draughty situation a considerably higher humidity is necessary for success in rearing from the egg—note the persistent mortality in the laboratory cupboard, which, in spite of its high percentage of humidity, gave scarcely any successful results (see Exp. *h*, Table XVI).

Moistening with either water or urine will mitigate a condition of extreme drought in which no larvae can survive for longer than a day or two. If liquid be applied in suitable quantities at regular intervals, breeding is possible. Moistening may, however, be easily overdone if applied injudiciously; there is some possibility that urine is a more effective moistening agent than water<sup>1</sup>.

<sup>1</sup> These moistening experiments were instituted as it was thought that the possibility of children urinating within a house or hut with earth floors, or damping of the floor from



From the experiments detailed in Table XV, the nature of the food supplied to larvae even when well grown is seen to be of importance. The fact that, in so humid a place as the cellar, there is so large a difference in the mortality of those nourished on rat faeces 80 % or B.S. rag 40 % respectively, suggests that the difference is really one of nutrition, and is not due to the mechanical difficulty of absorbing dry food from large masses. This point is further emphasised in Table XVI in the series carried out during May, June and July 1911 in the cellar and incubators 85 Wet and 75 Wet respectively, to test the relative values as food of B.S. rag, flea faeces and crushed rat faeces for *C. fasciatus*. The May series (in incubator 75 Wet) (Exp. a, Table XVI) consisted of a batch of young larvae from eggs laid and hatched under precisely similar conditions and then divided on the same date between the experiments. It will be noted that, while the flea faeces and B.S. rag batches showed a mortality of 29 % in both cases, the mortality with rat faeces as food was 100 %. A similar result is also apparent with experiments in the cellar on the 18th and 25th June (Exp. f, Table XVI) and in incubator 85 Wet on the 8th and 13th July (Exp. c, Table XVI), but in these cases the larvae under different dates were from separate layings.

When nourished on different diets, the speed with which larvae attain the cocoon stage varies considerably, conditions other than food being similar. The results obtained are somewhat contradictory, in some cases flea faeces and at others B.S. rag showing an advantage. These discrepancies are probably due to more than one cause. Firstly, the innate variability of the species must be taken into account and, secondly, the food supplied was not properly standardised. Flea faeces, which consist of the dejecta of *P. irritans* on the gauze coverings of the boxes in which they are kept, have possibly been given in varying quantities, it being no easy matter to gauge the exact quantity, and the B.S. rag has differed in quality, that is, in regard to the amount of blood on it. Three different cloths have been used since the commencement of the experiments, the second being decidedly inferior, while the last was much the best. It was not, unfortunately, anticipated that difference in quality of food would be of such great importance to

any cause might make all the difference between the extermination or survival of flea larvae during the dry season. The urination of rats, especially nestling rats, will certainly be an important factor in the relative scarcity or abundance of fleas in hot, dry climates. The factor of local moistening is of course applicable when fleas are reared on caged rats.

the larvae as now appears probable in the light of the experiments. The rationale of the matter seems to be that the best food gives the quicker development as well as the lowest mortality.

*P. irritans*. The results with this species largely resemble those obtained with *C. fasciatus* as regards conditions of temperature and humidity, but at the same time there is evidence of its being a hardier species, with greater ability to survive in hot and dry conditions. This is most clearly shown by its survival under cool, draughty conditions in the laboratory cupboard (see Exp. *g*, Table XXI), in which place hardly one larva of *C. fasciatus* was reared (see Exp. *h*, Table XVI). Further testimony is available from the hot Wet incubator series of the two species, a few individuals of *P. irritans* having been reared at a temperature of 93° F. (see Exp. *c*, Table XXI, Jan. 25 and Mar. 22, 1912).

The experiments of feeding on rat faeces and bran show that this species is also more adaptable in the matter of food. Probably the advance in human comfort and cleanliness will have gradually forced the larvae of *P. irritans* to abandon dependence they may once have had upon a diet having origin in the blood of their parents' host.

*X. cheopis*. A low percentage of humidity in the air is as fatal to this species as it is to *C. fasciatus* and *P. irritans*. Table XXV shows that the conditions in incubators 85 Dry and 75 Dry are quite as impossible for it as for the other species dealt with, although there is some hint (see warm cupboard under date of 4th September 1911, and incubator 85 Dry 28th August 1911 (Table XXV)) that the larvae have greater powers of endurance than those of *C. fasciatus* and *P. irritans*, and the evidence afforded by the tests applied to newly hatched unfed larvae is, on the whole, of a confirmatory character (see Table XII). On the other hand, the tests of transferring well grown larvae of the rat fleas from the cages to incubators to complete their metamorphosis are hardly in agreement (compare Table XIX with Table XV), and suggest that *C. fasciatus* is even better adapted to the exigence of a sudden rise in temperature than its congener. This is not, however, necessarily contradictory to the view that *X. cheopis* is better adapted for breeding under hot conditions than *C. fasciatus*, but rather that the latter is better able to resist a sudden change.

In Table XXV where comparison is made of the results obtained with *C. fasciatus*, *P. irritans* and *X. cheopis* under similar conditions it will be noticed that there is a lower mortality among *X. cheopis* reared in incubator 85 Wet and 75 Wet, which is reversed in the

cooler situations. It is evident that cold, or even cool, conditions are very fatal to *X. cheopis*. The cellar, laboratory cupboard and beehive experiments in Table XXV, as well as Table XIX, dealing with the transfer of last instar larvae from the cages, show clearly that *X. cheopis* is ill adapted to survive the cold of English autumn conditions.

*Ct. canis* (Table XXIV). Sufficient ova of this species were obtained from a dog's bed to permit of a small experimental test upon rearing the larvae under varied conditions of temperature and humidity.

The larvae were all hatched from eggs laid in incubator 75 Wet and then distributed in batches as usual. The species would appear to be very intolerant of extreme conditions during its active larval life and, so far as it is safe to judge from this one experiment, cool and draughty situations are most suitable for its development.

MITES. Attempts to feed larvae on oatmeal, or to use oatmeal in place of sand with other foods, failed with *C. fasciatus* (see Table XVI under date of 14th February), and similar attempts with *P. irritans* in incubator 75 Wet, 85 Wet, the cellar and laboratory cupboard, on a more elaborate scale, gave like results. In all cases the jars developed swarms of mites, and the larvae disappeared, leaving no trace of remains.

Two direct tests were made in order to see if the mites were responsible for the failure.

With *P. irritans* two tubes were prepared with the necessary sand and B.S. rag. Into one numbers of mites and 11 newly hatched larvae were placed; in the other a few mites only and 12 half-grown larvae; both tubes were kept in incubator 75 Wet. Within nine days all trace of the newly hatched larvae had disappeared. The tube containing the half-grown larvae produced nine fleas.

With *C. fasciatus* the following experiment was carried out: half-grown larvae were taken from the cages and a batch of 14 placed in each of two tubes prepared as follows: tube No. 1 contained sand, oatmeal and B.S. rag, and was quite free from mites; tube No. 2 contained exactly the same, but with the addition of swarms of mites. The quantity of food was large in order that there might be no question of scarcity of food. Twelve fleas were reared from tube No. 1 and twelve from tube No. 2, the average time in both cases being 19 days.

The factors of extermination would appear therefore to be the mites, but they appear to be inimical only to flea larvae while they are young; most probably the flea larvae are destroyed when moulting, as the latter are then too sluggish to resist or to get away.



16	"	25	"	Lab. cupboard	"	about 60	about '84	14	19	24	2	23
											2	25
											1	27
											2	29
											1	31
											1	34
											3	35
											2	42
											1	46
											1	49
											1	53
											2	59
30 Sept. '10		23	"	Lab. cupboard	"	about 60	about '84	--	15	35	1	26
											4	38
											2	40
											2	49
											2	54
											1	59
											2	75
											1	81
17 Aug. '10		13				up to Nov. approximate					Approximate	
19 "		9				60	'84				Shortest max. 26	
22 "		8									Average 45	
25 "		20				from Nov. to close of expt.		9	58	30	Longest min. 114	
15 Sept. '10		23	"	Lab. cupboard	B.S. Rag	max. min.						
19 "		10				54.9 44.5	'84					
Av. date, } 31 Aug. }		83										
29 Dec. '10		14	Tube	Beehive	B.S. Rag			11	9	36	1 took 148	
					Jan.	43.9 32.0					1 " 152	2 took 129
					Feb.	43.8 30.7					3 " 158	2 " 135
					March	52.5 33.3					1 " 131	30 Sept. '11 con-
					April	61.7 36.8					1 " 153	tained 2 living
					May	72.8 45.2					1 " 178	pupae, 2 empty.
					June	73.0 56.5					1 " 211	Remaining co-
					July	80.3 58.6					2 " 300	coons opened 16
					Aug.	80.2 57.6					1 " 267	Nov. '11, all were
					Sept.	75.3 48.0					1 " 267	empty
					Oct.	61.9 41.3						
9 Sept. '12		22	Card jar	Incubator 75 Dry	B.S. Rag	75.0	'52	--	--	100		1 larva attempted
"		22	"	" 93 Dry	"	92.0	'53	--	--	100		to spin

TABLE XVI\*. *Development of larvae, C. fasciatus. Influence of temperature, humidity and food supply upon larvae reared from egg; all eggs laid in incubator "75° Wet."*

520

Bionomics of Fleas

(a) Incubator 75° Wet.

Date	Tube	Box	Jar	Food	No. of larvae	Temperature	Humidity	Length of larval life to cocoon stage (in days)			Maximum number of days after hatching during which larvae were observed	No. of fleas reared	Mortality 100 %
								Min.	Aver.	Max.			
30 July '10	x	—	—	B.S. Rag	2	75	unrecorded	—	—	—	6	0	100
5 Aug. '10	x	—	—	"	4	75	.66	—	—	—	25	0	100
11 "	—	x	—	"	6	75	.70	—	—	—	3	0	100
24 Sept. '10	—	—	x	"	16	75	.72	20	21	22	—	8	50
30 "	—	—	x	Dead flies ( <i>M. domestica</i> )	6	—	—	9	12	17	—	6	Nil
16 Oct. '10	x	—	—	Rat faeces	15	74.3	.75+	—	14	—	—	1	93
19 "	—	x	—	B.S. Rag	9	74.3	.69+	—	—	—	—	0	100
30 "	x	—	—	"	15	74.2	.74	13	15	18	—	13	13
30 "	x	—	—	Rat faeces	13	74.2	.74	15	16	18	—	8	38
21 Dec. '10	—	—	—	B.S. Rag	28	74.3	.75	19	25	39	—	8	72
14 Feb. '11	x	—	x	+Oatmeal	10	74.4	.76	—	—	—	23	0	100
14 "	x	—	—	+Oatmeal and B.S. Rag	14	74.4	.76	—	—	—	23	0	100
14 "	x	—	—	+Oatmeal and flea faeces	12	74.4	.76	—	—	—	20	0	100
14 Mar. '11	x	—	—	Rat faeces crushed	26	75	.80	20	28	45	59	5	81
24 April '11	x	—	—	Powdered blood, no sand	8	74.7	.83	—	24	—	—	4	50
23 May '11	—	—	x	Flea faeces	28	75.8	.84	15	18	20	—	20	29
23 "	—	—	x	Rat faeces crushed	28	75.8	.84	15	17	20	34	0	100
23 "	—	—	x	B.S. Rag	28	75.8	.84	15	17	22	—	20	29
14 Nov. '11	—	—	x	Bran	22	75.0	.69	—	—	—	32	0	100

Moistening test (see also Table XVII).

(b) Incubator 75° Dry.

13 Jan. '11	x	Moistened	—	B.S. Rag	13	74.4	—	21	24	33	37	8	38
13 "	x	Not moistened	—	"	14	74.4	.75	—	—	—	35	0	100
21 Dec. '10	—	—	x	B.S. Rag	14	76.5	.41	—	—	—	—	0	100
15 Mar. '11	x	—	—	Rat faeces crushed	20	74.5	.49	—	—	—	—	0	100
28 Aug. '11	—	—	x	B.S. Rag	11	74.5	.61	—	—	—	—	0	100

No larvae seen after emergence  
 No larvae seen after placing in tube  
 1 larva seen after 2 days

(c) Incubator 85° Wet.

30 July '10	x	—	—	B.S. Rag	4	85	dropped from .80 to .68	—	—	—	5	0	100
4 Aug. '10	x	—	—	"	4	85	.74	—	—	—	—	4	Nil
10 "	—	x	—	"	1	85	.74	—	—	—	—	0	100
9 Sept. '10	—	x	—	Bran	3	85	.70	—	—	—	—	0	100
22 "	—	—	x	B.S. Rag	12	85	.70	—	—	—	—	5	59
13 Oct. '10	x	—	—	Rat faeces	9	84	.72	—	—	—	12	0	100
5 Nov. '10	x	—	—	"	14	84	.72	—	—	—	8	0	100
24 Dec. '10	—	—	x	B.S. Rag	16	84	.68	—	—	—	17	0	100
15 Mar. '11	x	—	—	Rat faeces crushed	26	84	.71	19	22	33	35	6	77
8 July '11	—	—	x	B.S. Rag	29	85.1	.83	9	14	23	—	22	24
8 "	—	—	x	Rat faeces crushed	28	85.1	.83	—	—	—	12	0	100
13 "	—	—	x	Flea faeces	28	85	.82	14	19	26	—	5	82
14 Nov. '11	—	—	x	Bran	22	85.2	.73 for 9 days	—	—	—	17	—	100
							.69 for 7 days						
							.66 for remainder						
26 Mar. '12	—	—	x	B.S. Rag	22	92.3	.67	—	—	—	20	—	100

(d) Incubator 85 Dry.									
24 Dec. '10	B.S. Rag	9	84.3	.60	—	—	—	No larvae seen after hatching	0
15 Mar. '11	Crushed Rat faeces	17	83.8	.59	—	—	—	2 days	100
28 Aug. '11	B.S. Rag	11	84.0	.64	—	—	—	No larvae seen after they were put into jar	100
(e) Warm Cupboard.									
			Max.	Min.					
6 Aug. '10	B.S. Rag	3	69	68	about .60	—	—	9	100
10 Oct. '10	"	10	74	69	about .60	—	—	None seen after emergence	100
22 "	Rat faeces	5	73	69	about .60	—	—	" "	100
12 Nov. '10	B.S. Rag	1	59	.53	—	—	—	2	100
29 Mar. '11	Rat faeces crushed	26	61.5	.62	—	—	—	No larvae seen after placing in tube	100
(f) Cellar.									
31 July '10	B.S. Rag	1	60	59	.93	—	34	—	1
4 Aug. '10	"	4	60	59	.93	30	33	34	25
14 "	"	1	59	58	.93	—	25	—	Nil
7 Oct. '10	"	19	48	47	.93	75	87	104	8
22 "	Rat faeces	3	48	47	.93	—	—	146	—
4 April '11	"	24	55.5	53.5	.92	69	99	114	100
25 June '11	" crushed	26	62.5	61.4	.93	—	—	73	91
18 "	Flea faeces	32	60.4	59.1	.93	33	37	45	92
25 "	B.S. Rag	27	61.1	59.9	.93	26	27	32	3
21 Aug. '11	"	14	{ Aug. 64.5	63.4	.94	{ 22	27	36	3%
21 "	Flea faeces	14	{ Sept. 60.6	59.5	.93	{ 22	31	45	7
(g) Beehive.									
			70	39					
10 April '11	Rat faeces crushed	19	70	39	—	—	—	12	100
(h) Laboratory Cupboard.									
			Approximate only until Nov. '10						
25 July '10	B.S. Rag	3	{		—	—	—	21	Nil
6 Aug. '10	"	2	{		—	—	39	45¶	—
12 "	"	4	{		33	34	39	—	Nil
18 "	Sweat soaked flannel	3	{		—	—	—	24	100
17 Sept. '10	Rat skin	4	{		—	—	—	62	100
27 "	B.S. Rag	4	{		60	.84	—	141	100
15 Oct. '10	Rat faeces	4	{		—	—	—	24	100
19 "	B.S. Rag	5	{		—	—	—	17	100
26 "	Rat faeces	several	{		—	—	—	94	100
29 Mar. '11	" crushed	26	56.3	45.8	.83	—	—	20	100
13 July '11	Flea faeces	28	72	64	.73	—	—	14	100
13 "	B.S. Rag	28	70	62	.74	—	—	Died off in less than 8 days	100
13 "	Rat faeces crushed	28	70	62	.74	—	—	" " 7 "	100

When two or more experiments bear the same date it may be taken that a batch of eggs or newly hatched larvae were divided.  
 \* Experiments given in Table XVI, up to and including those of 14 February, 1911, form a continuation of those made with ova set forth in Table II; data as to the corresponding cocoon stage will be found in Table XXVIII.

† The humidity was very low for a portion of the time, from the 21 to 24 Oct. 1910 it averaged .61 only.  
 ‡ The oatmeal developed swarms of mites. No sand was used.

§ 7 cocoons were opened and found to be empty, the larvae must have emerged again after spinning and died.

|| 8 fleas bred. 1 cocoon, opened 8 May 1911, contained a resting larva which died. Remaining cocoons empty (false cocoons); mortality is really nil.

¶ 1 cocoon opened 1 Jan. 1911 contained a living larva.

TABLE XVII\*. *Development of larvae, C. fasciatus. Influence of temperature and humidity upon larvae reared from egg; all eggs laid in incubator "75 Wet."*

(a) Incubator 75 Wet.												
Date	Tube	Box	Jar	Food	No. of Temperature larvae	Method of moistening	Length of larval life to cocoon stage (in days)			Maximum number of days after hatching during which larvae were observed	No. of fleas reared	Mortality
							Min.	Aver.	Max.			
1 Dec. '10	x	—	—	B.S. Rag	11	Water at start	—	—	—	22	0	100 <sup>9</sup> / <sub>10</sub>
6 "	x	—	—	"	18	"	—	—	—	17	0	100
8 "	x	—	—	"	14	"	—	—	—	No record	0	100
+28 "	Batch of eggs divided	x	x	"	23	Urine at intervals	15	20	22	40	3	87
+28 "					21	Water	15	22½	38	43	5	76
+4 Mar. '11	Batch of 72 eggs divided	x	x	"	13	Water 1 c.c. daily	—	10	—	14	1	92
+4 "					25	Urine "	—	17	—	27 (only 2 cocoons found)	12	52
(b) Incubator 75 Dry.												
8 Dec. '10	x	—	—	"	12	Water at start	—	—	—	No larvae seen after emergence	0	100
17 "	x	—	—	"	21	"	—	—	—	"	0	100
+3 Jan. '11	Batch of 42 eggs divided	x	x	"	12	Urine at intervals	—	—	—	"	0	100
+3 "					13	Water	—	—	—	"	0	100
+31 "	Batch of 61 eggs divided	x	x	"	21	Urine daily	—	—	—	"	0	100
+31 "					20	Water "	20	21	23	—	1	95
+28 Feb. '11	Batch of 60 eggs divided	x	x	"	7	1 c.c. Urine daily	—	—	—	20	0	100
+28 "					17	1 c.c. Water	—	—	—	11	0	100
+17 May '11	—	—	x	"	20	0.5 c.c. Urine daily	—	—	—	28	0	100
+17 "	—	—	x	"	20	0.5 c.c. Water	—	—	—	1	0	100
6 "	—	—	x	"	20	0.5 c.c. Urine "	—	—	—	No larvae seen after the 1st day	0	100
(c) Incubator 85 Wet.												
1 Dec. '10	x	—	—	"	12	Water at start	—	—	—	14	0	100
8 "	x	—	—	"	7	"	—	—	—	20	0	100
+30 "	Batch of 61 eggs divided	x	x	"	24	Urine at intervals	15	15	17	29	5	79
+30 "					16	Water	—	—	—	26	0	100
+24 Feb. '11	Batch of 72 eggs divided	x	x	"	19	Urine 1 c.c. daily	12	14	16	17	2	90
+24 "					21	Water "	12	16	19	40	2	91



(d) Incubator 85 Dry.

+25 Nov. '10	×	Batch of 41 eggs {	13	84	Urine at intervals	—	—	No larvae seen after hatching	0	100
+25 " "	×	divided	8	84	Water "	—	—	" "	0	100
1 Dec. '10	×	—	11	84	Water at start	—	—	" "	0	100
6 " "	×	—	15	83	" "	—	—	" "	0	100
8 " "	×	—	9	84	" "	—	—	" "	0	100
17 " "	×	—	19	84	" "	—	—	" "	0	100
+30 " "	×	Batch of 42 eggs {	20	84.9	Urine at intervals	—	—	" "	0	100
+30 " "	×	divided	15	85	Water "	—	—	No larvae seen after hatching	0	100
+27 Jan. '11	×	Batch of 39 eggs {	4	85	Urine daily	—	—	" "	0	100
+27 " "	×	divided	9	85	Water "	—	—	" "	0	100
+21 Feb. '11	×	Batch of 50 eggs {	hatching	84	1 c.c. Urine daily	—	—	" "	0	100
+21 " "	×	divided	unrecorded	84	1 c.c. Water "	24	25	37	2	—
6 May '11	—	—	20	84	0.5 c.c. Urine daily	16	18	23	12	40
15 " "	—	—	20	84	0.5 c.c. Water "	—	—	—	3	85

(e) Warm Cupboard.

9 Nov. '10	×	Batch of 64 eggs {	5	60	Urine at intervals	—	—	18	0	100
+3 Feb. '11	×	divided	12	65	Urine daily	—	—	20	0	100
+3 " "	×	—	19	63	Water "	—	95+	—	1	94
+8 Mar. '11	×	Batch of 70 eggs {	12	60.7	1 c.c. Urine daily	—	—	14	0	100
+8 " "	×	divided	16	61.2	1 c.c. Water "	28	30	37	10	38
5 May '11	—	—	20	62.6	0.5 c.c. Urine daily	24	25	4 larvae died after spinning	3	85
6 " "	—	—	20	64.6	0.5 c.c. Water "	31	36	2	4	80

\* The experiments given in Table XVII, up to and including those of 8 March 1911, form a continuation of those made with ova set forth in Table I; data as to the corresponding cocoon stage will be found in Table XXIX.

† against two experiments of the same date signifies that both were made with specimens from a single batch of ova or newly emerged larvae.

‡ One fully fed larva wandered about for 47 days before spinning cocoon.

(iii) LAGGING LARVAE. (See Table XXVI.) In addition to the ability of newly hatched flea larvae to exist for several days or weeks without food, some individuals of *C. fasciatus* were noticed to "lag" when they had attained their full growth, either finishing their larval feeding with extreme slowness or else waiting for considerable periods after they had finished feeding before spinning their cocoons.

It is possible that the larval period is not only lengthened by these practices in a few isolated instances, but that it is generally rendered more variable than would otherwise be the case.

When rearing from the egg, this phenomenon of "lagging" is not very noticeable owing to the varying speed attained in development by different individuals, but it becomes quite evident when a number of full sized larvae are taken from the cages. This was done between the end of January and the middle of March 1911 in order to obtain a number of newly emerged fleas to stock a new cage. About 120 larvae of *C. fasciatus* were put into a jar in incubator 75 Wet (temperature 74°F., humidity .74) on the 27th January, 1911. Several had spun their cocoons by the following day; many fleas emerged and were added to the new cage. On the 4th March it was decided to add a fresh batch of larvae from the cages to the same jar. When it was opened for this purpose some of the larvae put in on the 27th January were discovered to be still active after a period of 36 days, numbers of their fellows having in the meantime emerged as fleas. After putting in the second batch on the 4th of March, active larvae were observed in this jar until the 5th of May; allowing that these were all members of the second batch, it would show "lagging" on the part of some of them for varying periods up to 62 days.

A similar instance occurred with another batch of 100 larvae put into the same incubator on the 17th March; most of these spun at once, but stragglers were seen at intervals, the last being observed on the 19th April, over a month later.

A third series of 100 larvae, put into incubator 75 Wet on the 22nd March (temperature 75°F., humidity .74), showed the same phenomenon, the last active larva being seen on the 1st May, 49 days later. Advantage was taken of one of the "complex transference" cocoon experiments to follow the matter up with a little more detail (see Table XXVI), from which it appears that these lagging larvae are not simply pining individuals that subsequently die, for, as will be seen, most of them eventually spun cocoons and emerged.



TABLE XIX. *Development of larvae, X. cheopis. Influence of temperature and humidity upon larvae taken from the breeding cages when half to full grown.*

Date on which larvae were placed under test conditions	No. of larvae	Receptacle	Place	Food	Temp.	Humidity	No. of cocoons found	No. of fleas reared	Mortality	No. of days start to emergence	No. of days in cocoon	Remarks
30 Nov. '11	22	Card jar	Incubator 75	Wet	75.8	.65	21	22	nil	1-16 6-19 3-22 2-24 5-26 1-28 2-32 2-34	—	
30 Nov. '11	22	"	Incubator 75	Dry	74.7	.51	—	—	100%	—	—	No larvae seen alive after the start
30 Nov. '11	22	"	Incubator 92	Wet	92.4	.66	15	13	41	2-13 6-16 2-19 3-22	6-11 5-14 2-17	2 cocoons contained dried up fleas, remainder false cocoons
30 Nov. '11	22	"	Incubator 92	Dry	92.8	.57	13	10	54	3-13 5-16 2-19	6-11 4-14	2 cocoons contained dried up fleas, 1 a dried up larva, remainder false cocoons
5 Dec. '11	22	"	Cellar	"	48.7	.92	1	—	100	—	—	Cocoon opened 12 Apr. '12, found to contain living section of larva which subsequently died, 129 days
5 Dec. '11	22	"	Lab. cupboard	"	54.2	.84	1	—	at least 95	—	—	Cocoon opened, contained dead pupa
2 Apr. '12	22	"	Cellar	"	50.3	.93	1	—	100	—	—	Larvae dead by 12 Apr. Larvae dead in 2 days
2 Apr. '12	22	"	Lab. cupboard	"	60.6	.83	1	—	100	—	—	
2 Apr. '12	22	"	Beehive	"	64.1	.37.3	—	—	100	—	—	
2 Apr. '12	22	"	Warm cupboard	"	61	.61	—	—	100	—	—	
2 Apr. '12	22	"	Incubator 75	Wet	75.8	.79	21	19	13	1-18 3-21 9-25 2-27 3-35 1-38	1-13 1-14 10-17 4-19 2-21 1-20	
2 Apr. '12	22	"	Incubator 75	Dry	74.4	.56	—	—	100	—	—	Larvae dead in 2 days
2 Apr. '12	22	"	Incubator 92	Wet	93.1	.69	17	10	54	1-11 3-16 3-21 2-25 1-27	1-9 3-14 1-15 2-16 1-18 2-19	
2 Apr. '12	22	"	Incubator 92	Dry	93.7	.61	16	4	81	2-18 1-21 1-25	2-16 1-19 1-23	
9 Sept. '12	22	"	Incubator 75	Dry	75.0	.52	—	—	100	—	—	No larvae seen alive after the 2nd day
9 Sept. '12	22	"	Incubator 93	Dry	92.0	.53	—	—	100	—	—	No larvae seen alive after the start

TABLE XX\*. *Development of larvae, X. cheopis. Influence of temperature, humidity and food supply upon larvae reared from the egg; all eggs laid in incubator "75 Wet."*

Reports on Plague Investigations in India														527			
Date on which eggs were laid	Tube	Box	Jar	Food	Number	Temperature			Humidity	Length of larval life to cocoon stage (in days)			Max. number of days after hatching during which active larvae were observed	Number of Fleas	Mortality		
						max.	min.			Min.	Average	Max.					
Cellar.																	
6 June '11	×	—	—	B.S. Rag	6	June 58.1	56.7	.93	58	71	84	(2 cocoons)	1	83%			
3 July '11	—	—	×	Flea faeces	14	July 61.6	60.4	.93	44	46	65	(13 cocoons)	8	42			
3 July '11	—	—	×	Rat faeces crushed	14	Aug. 64.5	63.4	.94	37	42	44	(14 cocoons)	11	21			
21 Sept. '11	—	—	×	B.S. Rag	22	Sept. 60.6	59.5	.93	74 days				—	100			
						Oct. 56.3	54.8	.92	No larvae seen after 4 Dec.								
						Nov. 50.7	48.9	.91									
						Dec. 48.7	47.4	.92									
Incubator 85 Wet.																	
11 Aug. '11	—	—	×	B.S. Rag	13	84.4		.80	14	16	22		12	8			
11 Aug. '11	—	—	×	Flea faeces	11	84.3		.80	—	14	—		7	36			
4 Sept. '11	—	—	×	B.S. Rag	22	84.5		.74	12	13	16	(22 cocoons)	20	9			
Incubator 75 Wet.																	
4 Sept. '11	—	—	×	B.S. Rag	22	74.8		.76	14	19	21	(21 cocoons)	21	5			
Incubator 75 Dry.																	
28 Aug. '11	—	—	×	B.S. Rag	11	74		.61	—	—	—	No larvae seen after 1st day	—	—			
Incubator 85 Dry.																	
28 Aug. '11	—	—	×	B.S. Rag	11	84		.60	—	—	—	No larvae seen after 3rd day	—	—			
Warm Cupboard.																	
4 Sept. '11	—	—	×	B.S. Rag	22	68.1		.65	—	—	—	No larvae seen after 7th day	—	100			
Laboratory Cupboard.																	
4 Sept. '11	—	—	×	B.S. Rag	22	max. 65.7	min. 55.7	.77	(Lowest reading 40 on 22 Nov. and three times in Dec. before the 11th)						98 days	—	100
						Oct. 59.0	50.9	.84							No larvae seen after 11 Dec.		
						Nov. 54.9	46.1	.85									
						Dec. 52.7	44.3	.88									
Beehive.																	
20 Sept. '11	—	—	×	B.S. Rag	22	Sept. 75.3	48.0		(Lowest reading 28°F. on 26 Oct. '11, twice below 40 during Sep.)						37 days	—	100
						Oct. 61.9	41.3								No larvae seen after 27 Oct.		
Incubator 85 Wet.																	
14 Nov. '11	—	—	×	Bran	22	10 days at 85.2		.73							15 days	2	90
						7	75.0	.69									
Incubator 75 Wet.																	
22 Nov. '11	—	—	×	Bran	22	75.0		.69							20 days	18	18

\* The continuation of these experiments through the cocoon-stage will be found in Table XXXIV.

TABLE XXI\*. *Development of larvae, P. irritans. Influence of temperature, humidity and food supply upon larvae reared from the egg.*

(a) Incubator 75 Wet.													
Date on which eggs were laid	Tube	Box	Jar	Food	No. of larvae 7 eggs, no record of hatching	Temp. 75	Humidity Unrecorded	Length of larval life to cocoon stage (in days)			Max. number of days after hatching during which active larvae were observed	No. of fleas reared	Mortality
								Min.	Av.	Max.			
13 July '10	×	—	—	Rat faeces	7	75	Unrecorded	15	16	18	17	2	—
19 " '10	×	—	—	Dead flies†	4	75	"	12	15	17	17	2	50%
11 Aug. '10	×	—	—	B.S. Rag	7	75	.66	22	23	26	—	4	43
1 Oct. '10	—	—	×	"	5	74.3	.72+	—	12	—	13	1	80
10 " '10	—	—	×	"	45	74.3	.78+	—	—	—	6	0	100
14 " '10	—	×	—	Bran	15	74.3	.74+	—	—	—	No larvae seen after hatching	0	100
15 Feb. '11	×	—	—	{ Oatmeal§	17	74.4	.76	—	—	—	23	0	100
15 " '11	×	—	—	{ Oatmeal and flea faeces§	6	74.4	.76	—	—	—	20	0	100
15 " '11	×	—	—	{ Oatmeal and B.S. Rag§	12	74.4	.76	—	—	—	23	1	92
27 Mar. '11	×	—	—	Rat faeces crushed	13	75	.80	14	19	23	—	10	23
24 Apr. '11	×	—	—	Powdered dry blood, no sand	7	74.7	.83	—	—	—	18	0	100
9 June '11	—	—	×	B.S. Rag	24	75.7	.85	10	15	26	—	21	13
9 " '11	—	—	×	Flea faeces	24	75.8	.85	15	21	46	—	5	79
18 Oct. '11	—	—	×	B.S. Rag	22	74.4	.73	13	16	23	—	16	27
(b) Incubator 75 Dry.													
4 June '11	—	—	×	B.S. Rag	20	78	.63	—	—	—	No larvae seen after start	0	100
9 June '11	—	—	×	Flea faeces	24	75	.53	—	—	—	"	0	100
22 Aug. '11	—	—	×	B.S. Rag	26	75.7	.59	—	—	—	3 larvae seen on 2nd day, none afterwards	0	100
(c) Incubator 85 Wet.													
24 July '10	×	—	—	B.S. Rag	13 eggs, no record of hatching	about 85	Doubtful record, varies from .80 to .68	—	12	—	—	1	—
6 Aug. '10	×	—	—	"	12 eggs	85	.74	—	9	—	—	1	—
26 Sept. '10	—	—	×	Flea faeces	23	85	.70	9	15	22	27	4	83
14 Oct. '10	—	×	—	Bran	7	84	.72	No cocoons found	—	8	8	2	72
29 Mar. '11	×	—	—	Rat faeces crushed	15	84	.71	9	12	15	—	8	47
6 June '11	—	—	×	B.S. Rag	26	84	.80	8	14	24	—	16	38
7 " '11	—	—	×	Flea faeces	20	84	.80	12	14	17	—	8	60
14 Nov. '11	—	—	×	Bran	22	85.2	.73	—	—	—	15	1	95
5 Jan. '12	—	—	×	B.S. Rag	24	92.6	.66	11	12	15	—	1	95
22 Mar. '12	—	—	×	"	22	92.3	.67	10	11	12	—	9	59
(d) Incubator 85 Dry.													
6 June '11	—	—	×	B.S. Rag	26	85	.61	—	—	—	No larvae seen after start	0	100
7 " '11	—	—	×	Flea faeces	20	85	.50	—	—	—	"	0	100
22 Aug. '11	—	—	×	B.S. Rag	26	83.8	.63	—	—	—	"	0	100

(e) Warm cupboard.

22 Sept. '10	Flea faeces	×	—	3	max. min.	74 61	Between 50 & 60	—	—	No larvae seen after emergence	0	100
6 Oct. '10	B.S. Rag	—	—	23 or more	76 65	" "	" "	—	—	" "	0	100
18 "	Bran	—	×	4	74 70	" "	" "	—	—	" "	0	100
12 June '11	Flea faeces	—	—	20	Dry 65.0	" "	" "	—	—	No larvae seen after start	0	100
6 "	B.S. Rag	—	×	26	73.0	.62	" "	—	—	" "	0	100

(f) Cellar.

6 Oct. '10	B.S. Rag	×	—	9	max. min.	48 47	.91—.93	76	106	137	196	2 cocoons	100
14 "	Bran	—	×	11	48 47	.91—.93	.93	122	162	202	212	2	82
3 June '11	B.S. Rag	—	—	24	June 58.1 56.7	.93	.93	{ 48	54	73	—	16	33
12 "	Flea faeces	—	—	28	July 61.6 60.4	.93	.93	{ 39	50	78	92	16	42
					Aug. 64.5 63.4	.94	.94						
					Sept. 60.3 59.5	.93	.93						
11 Aug. '11	Flea faeces	—	—	35	Aug. 64.5 63.4	.94	.94	{ 26	37	55	—	23	34
11 "	B.S. Rag	—	—	35	Sept. 60.6 59.5	.93	.93	{ 32	42	65	1 flea took 272 days from start	17	51
					Oct. 56.3 54.8	.92	.92						
					Nov. 50.7 48.9	.91	.91						
					Dec. 48.7 47.4	.92	.92						
					Jan. 47.2 45.9	.92	.92						
					Feb. 45.8 44.6	.92	.92						
					Mar. 49.1 48.1	.93	.93						
					Apr. 50.3 49.1	.93	.93						
					May 54.2 53.2	.93	.93						

(g) Laboratory cupboard.

13 July '10	Rat faeces	×	—	2	4 eggs, no record of hatching	—	—	37	—	—	—	1	50
29 "	Dust from sleeping bag	—	×	2	—	—	—	—	—	—	26	2	—
29 "	Old carpet	—	×	1	—	—	—	—	—	—	32	0	100
1 Aug. '10	Flea faeces	×	—	9	60†	.84†	.84†	35	39	44	—	1	Nil
11 "	B.S. Rag	×	—	5	—	—	—	17	32	39	44	6**	34
14 "	Dust from sleeping bag and a few spots of flea faeces on gauze	—	×	10	—	—	—	34	50	62	—	1	80
20 "	Flea faeces	—	×	17	—	—	—	65	85	100	—	5	50
1 Oct. '10	B.S. Rag	—	×	8	—	—	—	81	84	87	223	11	36
10 "	Bran	—	×	24	max. min.	.77	.77	39	47	54	—	7	71
3 June '11	B.S. Rag	—	×	28	69.0 61.1	.77	.77	30	39	58	—	13	54
12 "	Flea faeces	—	×										

(h) Beehive.

6 "	B.S. Rag	×	—	26	79 56	—	—	—	—	—	No larvae seen after start	0	100
12 "	Flea faeces	×	—	20	69.5 50.5	—	—	—	—	—	2	0	100

\* Experiments given in Table XXI up to and including those of 15 February 1911, form a continuation of those made with ova set forth in Table V; data as to the corresponding cocoon stage will be found in Table XXXVI.

† Humidity ran very low for a portion of the time, from 21—24 October it averaged .61 only.

‡ Larvae came out, wandered about and failed to pupate.

§ Oatmeal developed swarms of mites.

¶ Temperature and humidity approximate only to Nov. 1910, see actual records after this date in relation to extended larval life.

\*\* 1 cocoon opened 1 Jan. 1911 contained living flea.

TABLE XXII. Development of larvae, *P. irritans* reared from the egg. Influence of varied situation (temperature and humidity), nature of food supply being kept constant; in each experiment all eggs taken from the same batch.

Date (a) on which eggs were laid	Place	Receptacle	Food	Number of larvae	Temperature	Humidity	Length of life to cocoon stage (in days)			Maximum number of days after hatching during which larvae were observed	Number of fleas reared	Mortality 100 %
							Min.	Aver.	Max.			
14 Oct. '10 46 eggs divided	Incubator 75 Wet	Box	Bran	15	Dry 74.3	.74	—	—	—	—	0	—
	Cellar	"	"	11	max. min. 48 47	from 21 May .61 only	122	162	202	212	2	82
11 Aug. '10 27 eggs divided	Incubator 75 Wet	Tube	B.S. Rag	7	Dry 75	.66	22	23	26	—	4	43
	Lab. Cupboard	"	"	9	approx. 60	.84	35	39	44	44	6	34
(b) on which larvae were placed under test conditions												
6 June '11	Incubator 85 Dry	Jar	"	26	85	.61	—	—	—	No larvae seen after start	0	100
	" 85 Wet	"	"	26	84.1 max. min. 60.8 59.5	.78	8	14	24	—	16 (2 cocoons empty)	38
3 June '11	Cellar	"	"	24	60.8 59.5	.93	48	54	73	—	16	42
	Lab. Cupboard	"	"	24	68.4 60.1	.77	39	47	54	—	7	71
6 June '11	Warm Cupboard	"	"	26	73 max. min. 79 56	.62	—	—	—	No larvae seen after start	0	100
	Beehive	"	"	26	79 56	—	—	—	—	" "	0	100
7 June '11	Incubator 85 Wet	"	Flea faeces	20	84	.79	12	14	17	—	8 (1 cocoon opened empty)	60
	" 85 Dry	"	"	20	85	.49	—	—	—	No larvae seen after start	0	100
9 June '11	" 75 Wet	"	B.S. Rag	24	75.7	.85	10	15	26	—	21	13
	" 75 Wet	"	Flea faeces	24	75.8	.85	15	21	46	—	5	79
	" 75 Dry	"	"	24	75	.55	—	—	—	No larvae seen after start	0	100
	Cellar	"	"	28	—	—	—	—	—	—	16	42*
12 June '11	Lab. Cupboard	"	"	28	max. min. 69.0 61.1	.77	30	39	58	—	13	54
	Warm Cupboard	"	"	20	65 max. min. 68.6 46.3	.59	—	—	—	No larvae seen after start	0	100
	"	"	"	20	68.6 46.3	—	—	—	—	2	0	100
	Beehive	"	"	20	68.6 46.3	—	—	—	—	—	0	100

\* For conditions of temperature and humidity see record in Table XXI.



TABLE XXIII. Development of larvae C. gallinae reared from the egg; eggs obtained from adults kept in incubator "75 Wet" and fed daily on human blood.

Date	Receptacle	Food	Place	Number	Temp.	Humidity	Length of larval life to cocoon stage (in days)			Mortality	Duration of cocoon period	No. of fleas reared
							Min.	Aver.	Max.			
24 March '11	Tube	Dust from nest and flea faeces	Incubator 75 Wet	16	Mar. 74.3 April 75.0 May 74.9	.76 .78 .85	13	14	21	Nil	1 in 6 days 4 in 9 days 2 in 11 days 1 in 12 days 1 in 13 days 1 in 14 days 1 in 16 days	11 5 larvae were taken as specimens

TABLE XXIV. Development of larvae Ct. canis. Influence of temperature and humidity; eggs obtained from a dog's bed and hatched in incubator "75 Wet."

Date	Tube	Box	Jar	Food	Number	Temperature	Humidity	Length of larval life to cocoon stage (in days)			Max. number of days after hatching during which active larvae were observed	No. of fleas	Mortality
								Min.	Aver.	Max.			
9 May '12	—	—	×	B.S. Rag	22	75.6	.86	12	13	19	—	8	63 %
"	—	—	×	"	22	95.1	.75	—	11	—	—	0	100
"	—	—	×	"	22	93.3	.58	—	—	—	5 days	0	100
"	—	—	×	"	22	75.0	.66	—	—	—	1 day	0	100
"	—	—	×	"	22	*—	—	49	61	142	52 days	2	91
"	—	—	×	"	22	*—	—	35	39	42†	38 days	13	40
"	—	—	×	"	22	max. 74.3 min. 54.3	—	—	—	—	3 days	0	100
"	—	—	×	"	22	May 39.3 June 40.9 July 43.6	.95 .99 .95	—	—	—	75 "	0	100

\* See page 461 for records of temperature and humidity, May to August 1912.  
† 2 fleas emerged 15 July '12; 2 living fleas were taken out of cocoon on 6 Sept.; 9 living fleas were taken out of cocoon on 1 Oct. 1912.

TABLE XXV\*. Development of larvae, comparing influence of temperature and humidity upon larvae of the three species *X. cheopis*, *P. irritans*, and *C. fasciatus*.

Date 1911	Receiptacle	Species	Place	Food	Number	Temperature	Length of larval life to cocoon stage (in days)			Max. number of days after hatching during which active larvae were observed	No. of fleas	Mortality 100 %
							Humidity	Min.	Aver.	Max.		
28 Aug.	Jar	<i>X. cheopis</i>	*Incubator 85 Dry	B.S. Rag	11	84	.60	—	—	—	—	100
29 "	"	<i>P. irritans</i>	"	"	11	84	.60	—	—	—	—	100
28 "	"	<i>C. fasciatus</i>	"	"	11	84	.60	—	—	—	—	100
28 "	"	<i>X. cheopis</i>	Incubator 75 Dry	"	11	74	.61	—	—	—	—	100
29 "	"	<i>P. irritans</i>	"	"	11	75	.58	—	—	—	—	100
28 "	"	<i>C. fasciatus</i>	"	"	11	74.6	.59	—	—	—	—	100
4 Sept.	"	<i>X. cheopis</i>	Incubator 85 Wet	"	22	84.5	.74	12	13	16	20	9
4 "	"	<i>P. irritans</i>	"	"	22	84.7	.77	10	12	19	19	13
11 "	"	<i>C. fasciatus</i>	"	"	21	84.9	.77	9	13	19	14	33
4 "	"	<i>X. cheopis</i>	Incubator 75 Wet	"	22	74.8	.76	14	19	21	21	5
4 "	"	<i>P. irritans</i>	"	"	22	74.9	.75	12	18	30	17	22
14 Oct.	"	<i>C. fasciatus</i>	"	"	22	74.4	.71	13	20	31	20	9
4 Sept.	"	<i>X. cheopis</i>	Warm Cupboard	"	22	68.1	.65	—	—	—	—	—
4 "	"	<i>P. irritans</i>	"	"	22	69	.71	—	—	—	—	—
6 "	"	<i>C. fasciatus</i>	"	"	22	67	.66	—	—	—	—	—
						max. min.						
4 "	"	<i>X. cheopis</i>	Lab. Cupboard	"	22	Sept. 65.7	.77	—	—	—	—	100
5 "	"	<i>P. irritans</i>	"	"	22	Oct. 59.0	.84	45	62	83	5	77
5 "	"	<i>C. fasciatus</i>	"	"	22	Nov. 54.9	.85	56	62	67	—	81
						Dec. 52.7	.88	—	—	—	—	—
20 "	"	† <i>X. cheopis</i>	Beehive	"	22	Sept. 75.3	—	—	—	—	0	100
19 "	"	<i>P. irritans</i>	"	"	22	Oct. 61.9	—	—	—	—	—	100
19 "	"	<i>C. fasciatus</i>	"	"	22	Sept. 60.6	.93	83	88	100	5	68
21 "	"	‡ <i>X. cheopis</i>	Cellar	"	22	Oct. 56.3	.92	37	52	63	—	100
25 "	"	<i>P. irritans</i>	"	"	22	Nov. 50.7	.91	37	48	70	4	81
25 "	"	<i>C. fasciatus</i>	"	"	22	Dec. 48.7	.92	37	48	70	9	36

\* In compiling this table, the records for *X. cheopis* are all taken from Table XX and two records for *C. fasciatus* (those in incubators 85 Dry and 75 Dry) from Table XVI. The remaining figures given for *C. fasciatus* and those for *P. irritans* are the results of special experiments not quoted elsewhere.

† 3 July, 1 cocoon opened contained resting larva; 6 Sept., 2 cocoons opened contained resting larvae, 367 days; 11 Sept., 1 cocoon opened contained resting larva, 372 days.

‡ No larvae seen subsequently to 27 Oct. 1911. Lowest reading 28° F. on 26 Oct. 1911. Thermometer was on two occasions below 40° F. during Sept. § Last flea emerged 15 June 1912; 4 July, a cocoon opened contained resting larva which pupated on 3 Sept. 1912, 350 days; 11 Sept. 1912, 1 cocoon opened contained a living pupa, 358 days.

¶ No larvae seen subsequently to 4 Dec. 1911.

\*\* 6 fleas emerged up to 6 Sept. 1912; 3 fleas emerged 11 Sept. 1912 upon the cocoons being disturbed; cocoons opened 11 Sept. 1912, 5 living pupae, 2 dead pupae, 2 empty.

¶ Last flea emerged 16 May 1912.

TABLE XXVI. *Lagging. Full grown larvae, C. fasciatus taken from the cages.*

Date	Number	Place	Receptacle	Temperature	Humidity	Active larvae observed	Cocoons found
11 April '11	26	Incubator 75	Wet Tube	74.7	.87	Several 5 May = 24 days	12 in 2 days
						8 " = 27 "	3 in 4 "
						10 " = 29 "	1 in 5 "
						Two 12 " = 31 "	1 in 31 "
							2 in 34 "
"	26	Beehive	Tube	max. 65 min. 39	—	One 22 " = 41 "	Only one cocoon found within 8 days
26 April '11	Several	Incubator 75	Wet Tube	74.5	.83	Several 8 " = 12 "	No record. Pupae taken out of cocoons for specimens
						10 " = 14 "	
						12 " = 16 "	
						15 " = 19 "	
11 April '11	26	Cellar	Tube	max. 48.5 min. 46.6	.91	18 " = 37 "	1 in 2 days
				May 53.9 52.6	.92	One 8 June = 58 "	3 in 6 "
				June 58.1 56.7	.93	15 " = 65 "	9 in 11 "
							3 in 14 "
							1 in 16 "
							2 in 42 "
							5 in 46 "
							1 in 58 "
							1 in 76 "

## 3. COCOONS (Tables XXVIII to XLIV).

i. *Influence of temperature, humidity and previous history upon duration of this period.*

In the life history of the flea the cocoon stage is the period in which it is most independent of external conditions of temperature, humidity, etc. and in least danger from the attacks of enemies. It is, therefore, not surprising to find that there tends to be an accumulation of individuals in this stage, from which the active adult population is recruited when suitable conditions offer. In the cocoon period, as in other stages, external conditions and individual idiosyncrasy combine to produce an inextricably interwoven condition of affairs. An attempt was made to disentangle the several strands by means of three different series of experiments with each species:

a. "Continuous" experiments in direct continuity with previous egg and larval experiments:—The cocoons were kept until the emergence of fleas in the same situations as the eggs were laid and hatched and the larvae reared. In the case of *C. fasciatus* these experiments are to be found in Tables XXVIII and XXIX, the corresponding observations on larval and egg stages being given in Tables XVI, XVII and I and II respectively. The cocoon observations for *X. cheopis* set forth in Table XXXIV correspond to Table XX (larvae) and those for *P. irritans* in Table XXXVI to Tables XXI (larvae) and V (ova). In this series the observations upon cocoons are necessarily more limited than the corresponding ones dealing with both eggs and larvae, for in many cases the drastic conditions to which the last-named were subjected left no survivors to undergo a cocoon stage. In nature it is usual for larvae to develop and to spin cocoons before the onset of climatic conditions, which would be fatal to them in the free state.

b. "Simple transference" experiments in which cocoons were obtained as follows:—In case of *C. fasciatus* and *X. cheopis* full-grown larvae were taken from the breeding cages and placed in card jars with sand and B.S. rag. The jars were all put into incubator 75 Wet and the larvae allowed to spin. The cocoons as found were transferred to glass bottomed boxes and these were buried in sand and placed in the different situations investigated. Table XXXI deals with *C. fasciatus* and Table XXXV with *X. cheopis*. In Table XXXVIII are given the results of a series of experiments with *P. irritans*, arranged to correspond

as nearly as possible with those of the other two species. In this instance, however, the larvae were reared until full grown in incubator 75 Wet. In this series of experiments the influence of varied conditions prior to spinning is eliminated as far as possible, and the results afford a comparison, uncomplicated by other circumstances, of the influence of varied external conditions upon the cocoon stage itself.

c. "Complex transference experiments" in which cocoons were maintained under conditions which were the opposite of those under which they were spun. For example, a batch of larvae taken from the breeding cages (in case of rat fleas) or reared in incubator 75 Wet (in the case of *P. irritans*) was placed to spin in incubator 85 Dry, after which the cocoons were transferred to the cellar. In other cases, cocoons spun in the cellar were maintained till emergence of the adult in incubator 85 Dry, and so on. In the tables dealing with this third series (XXXII in case of *C. fasciatus* and XXXIX in case of *P. irritans*) the number of days recorded as being passed in the cocoon stage is approximate<sup>1</sup> only, as the cocoons were not all changed into their new quarters on the same day.

In some of the experiments of the "continuous" series during 1910 and the early months of 1911 discrepancies will be noted between the numbers of cocoons investigated and the fleas recovered. These differences came about owing to the habit the flea larva possesses of sometimes forsaking its cocoon and wandering away to seek a more suitable situation. The deserted cocoons do not differ in any outward respect from full ones and constitute a source of possible error in estimating the mortality at the cocoon stage. So soon as this habit was discovered, deserted cocoons were subjected to careful examination at the close of an experiment, in order to determine whether they were really empty or contained the cast larval skin. The mortality in all but the earliest experiments is based upon the number of cocoons in which actual remains of dead larvae, pupae or fleas were found in the cocoon. In a few of the 1910 experiments, however, made before this routine examination had been instituted, the calculated percentage of failures in the cocoon stage may be too high owing to the inclusion of deserted cocoons. In the "continuous" series the error will consist in attributing too great mortality to the cocoon stage and too little to the larval stage.

<sup>1</sup> For instance, if two cocoons were transferred to the test situation on 1st January and two more on the 10th January, and one flea emerged on February 1st, it would be reckoned to have spent 30 days in the cocoon.

In the second and later series of experiments there is possibly an erroneous estimate of the number of cocoons experimented with. On the whole it was considered better to underestimate the mortality rather than overestimate it and therefore, in making the calculation, empty cocoons that retained no trace of a tenant were disregarded. They are referred to in the tables as "false" cocoons, and in some cases, where the numbers seemed unduly large, the percentage of mortality has been put down as doubtful. This caution is thought necessary because in hot and dry situations there is a possibility that the larva may leave its cocoon and failing to find a more suitable situation may break up into unrecognizable fragments among the sand and other debris from the cocoons.

Further sources of error in obtaining cocoon statistics are the possibility of two larvae spinning their cocoons against one another and the rare chance of a double cocoon being formed. In only one or two instances, however, has the recorded number of cocoons been exceeded by the joint number of fleas emerging and of remnants found. There is still the possibility that in these cases a wrong count was made.

*P. irritans*<sup>1</sup>. The results of the "continuous" series of experiments with this species are given in Tables XXXVI and XXXVII. In the "simple transference" (Table XXXVIII) and "complex transference" experiments (Table XXXIX) the larvae used were reared until full grown in incubator 75 Wet.

For full comprehension of the degree of variability displayed by the cocoons of this species in their reaction to external conditions, it is necessary to study in detail the four Tables referred to above, but the following paragraphs may be found useful in giving a general review of the results obtained.

The influence of high temperature in shortening the duration of the cocoon stage may be seen in the following summary compiled from Table XXXIII.

Experiments, in which the average humidity was between .7 and .9.

Temperatures	Average humidity	Number of cocoons	Average duration of cocoon stage
83.9—85.0	} from .7 { to .9 {	77	11.9
74.4—76.1		78	17.2

<sup>1</sup> The experiments with *P. irritans* are discussed first because with this flea the nature of the reaction to temperature and humidity appears to be less complex than in the case of *C. fasciatus*.

When the range of temperature was at a lower level, the divergence in cocoon period corresponding to difference of temperature was much greater: compare experiments in incubator 75 Dry, 15th April, 1911 and warm cupboard, 19th April, 1911 (Table XXXVIII).

Temperature	Average humidity	Number of cocoons	Average duration of cocoon stage
75·2	·50	4	14 days
63·6	·60	5	44 „

A still greater contrast is shown in the experiment of 11th August, 1910 (Table XXXVII) in which the average duration of the cocoon stage is compared in the case of individuals reared throughout in incubator 75 Wet and the laboratory cupboard respectively.

Temperatures	Average humidity	Number of cocoons	Average duration of cocoon stage
75° F	·60	4	12 days
53 (mean)	·83	6	116 „

As regards humidity it is possible that the increase of ·10 in the second instance given above may be a factor in extending the cocoon period; it is probable, however, that it is unimportant and masked by the normal variation of the individual cocoons. A comparison of the different experiments in Table XXXVIII shows somewhat contradictory evidence in regard to the effects of humidity. For example, in the case of experiments in incubators 85 Dry and 85 Wet, the shorter duration of the cocoon period corresponds to the higher humidity, while a like comparison of the results in 75 Wet and 75 Dry shows that at this temperature the shorter duration usually occurs under the drier conditions. Further, if we compare the records of 75 Wet among themselves we find longer periods corresponding to lower humidity. The conclusion to be drawn is that, in comparison with humidity, temperature and individual variation are the important factors.

There is some evidence suggesting that change to cooler conditions about the spinning period is important in lengthening the cocoon stage, apart from the temperature during this stage<sup>1</sup>. At least this is a plausible explanation of the divergent results obtained with broods reared in the laboratory cupboard from the egg stage onwards and those transferred from a warm incubator. Two batches of newly hatched larvae were put into the laboratory cupboard on the 3rd and 12th June

<sup>1</sup> Work by Merrifield on the Seasonal Dimorphism of some species of Lepidoptera shows that an otherwise latent tendency to lengthen the pupal period may be made in response to a change of temperature chiefly if not only at some one time in the larval life. *Experimental Entomology. Factors in Seasonal Dimorphism*, F. Merrifield, F.E.S. Extrait I<sup>er</sup> Congrès International d'Entomologie, Bruxelles, 1910.

1911. They had reached the cocoon stage by the 12th July and 12th August respectively, and both batches had an average cocoon period of 17 days, see Table XXXVI. On the other hand in a batch of 41 cocoons transferred from incubator 75 Wet to the Laboratory cupboard on the 30th May 1911 (Table XXXVIII (c)) the average cocoon period of the 32 specimens that emerged, was 53 days. In the latter case the cocoon period began earlier than with the batches put in on the 3rd and 12th June, it continued over the same period, and, in the case of some individuals, was prolonged later. It seems probable that the explanation of this result is that *P. irritans* has a seasonal habit<sup>1</sup>, passing the cold months in the cocoon period if possible. A fall of temperature of any moment, as at the approach of autumn, occurring during the late larval or early cocoon stage, brings into action some inherited tendency to lengthen the cocoon period. The length of this stage is due to individual variation as well as to the actual conditions experienced. In this connection it should also be noticed that *change* from a lower to a higher temperature near the date of spinning was also followed by a marked lengthening of the cocoon period.

Below are summarised two interesting experiments from Table XXXIX. In Exp. (a), under date 27th April, 1911, the cocoons were spun in the cellar and shortly afterwards transferred to incubator 85 Dry; in Exp. (b) (May 2nd, 1911) the cocoons suffered a less drastic change: spun in the laboratory cupboard, they were afterwards moved to incubator 75 Dry. In this case the average duration of the cocoon period was only 17 days, whereas in Exp. (a) it was 58 days.

	Temperature at spinning	Humidity at spinning	Transferred to temperature	Humidity	Average duration of cocoon period
(a)	54.1	.92	84.1	.6	58 days
(b)	58.0	.78	75.3	.53	17 „

The following experiments also give good illustration of the influence of a sudden fall in temperature in lengthening the cocoon period. In the "complex transference" experiment under date 2nd May, 1911, Table XXXIX, cocoons were spun in incubator 85 Dry, at a temperature of 84.3 F. and average humidity .59; they were then transferred to the cellar with a mean temperature of about 57 F. during the course of the experiment. The average cocoon period was 48 days. In one

<sup>1</sup> There is perhaps some indication of a seasonal habit in the fact that the November cocoons reared late in the autumn tend to show a higher mortality than in the spring and summer (see Table XXXVIII, experiments in incubator 75 Wet).



of the continuous experiments, the material was kept in the cellar throughout from the egg stage (Table XXXVI under date 3rd June, 1911) at a mean temperature of about 62° F., humidity .90. In this case the individuals passed on an average only 19 days in the cocoon. The inference appears to be that the susceptibility to sudden fall in temperature is, or may be, continued into the early period of cocoon life.

*C. fasciatus*. With *C. fasciatus* we find a much more complex state of affairs. Individual variation occurs as in the case of *P. irritans*. There is also conclusive evidence of lengthy resting within the cocoon under conditions of high as well as low temperature and over a wide range of humidity. This may be a simple temperature effect or due to change in temperature or both.

A close but general survey of the records obtained convinced me that there was evidence both of an aestivating and an hibernating habit in this species. Extremes both of heat as well as cold produce an effect which is partly of a direct nature and partly, perhaps, a stimulus which calls into action an inborn predisposition to prolonged rest within the cocoon under unfavourable conditions. As was found in the case of *P. irritans*, it is the *changes* in temperature which appear to be the controlling factor.

This suggestion, in spite of some contradictory evidence, is supported by a comparison of the following results, which moreover indicate that the more acute the change the greater the response. For example, in the "simple transference" experiments, detailed in Table XXXI, taking place in incubators 85 Wet and 85 Dry (Exp. (a) 30th Jan. 1911 and Exp. (b) 24th March, 1911) there is a break in the record of emergences between about the 25th day and the 70th day after the cocoons were transferred. The results of the same experiments are graphically expressed in Charts 7 and 8, in which the double period of emergence is clearly shown.

If I am not mistaken, this same feature may be observed in the other experiments (see, for example, Tables XXXI and XXXII and Chart 9) though not so clearly, and the suggestion is that we have here traces of an inherent discontinuity in the development of this species which in nature may favour a spring or autumn emergence of adults. It is an interesting question whether the individuals whose emergence is delayed by heat are the same as those whose emergence may be delayed by cold, or if the influence of extremes of temperature varies in different individuals. While there is no direct evidence

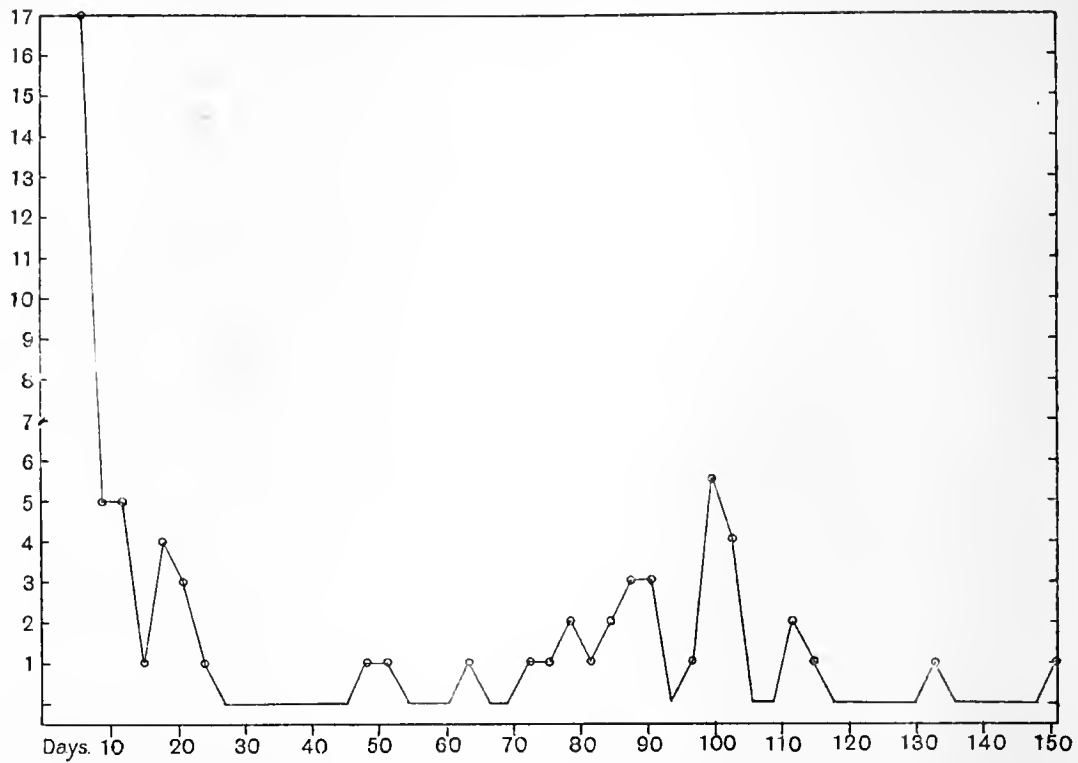


Chart 7. Emergence of *C. fasciatus* from cocoons in Incubators 85 Dry and 85 Wet (see Exps. Jan. and March 1911, Table XXXI, Nov. 1910 and April 1911, Table XXXII).

Temperature approximately 84° F.

Humidity average between .60 to .76.

Vertical numbers = units of emergence. Horizontal numbers = time in days (3 day units).

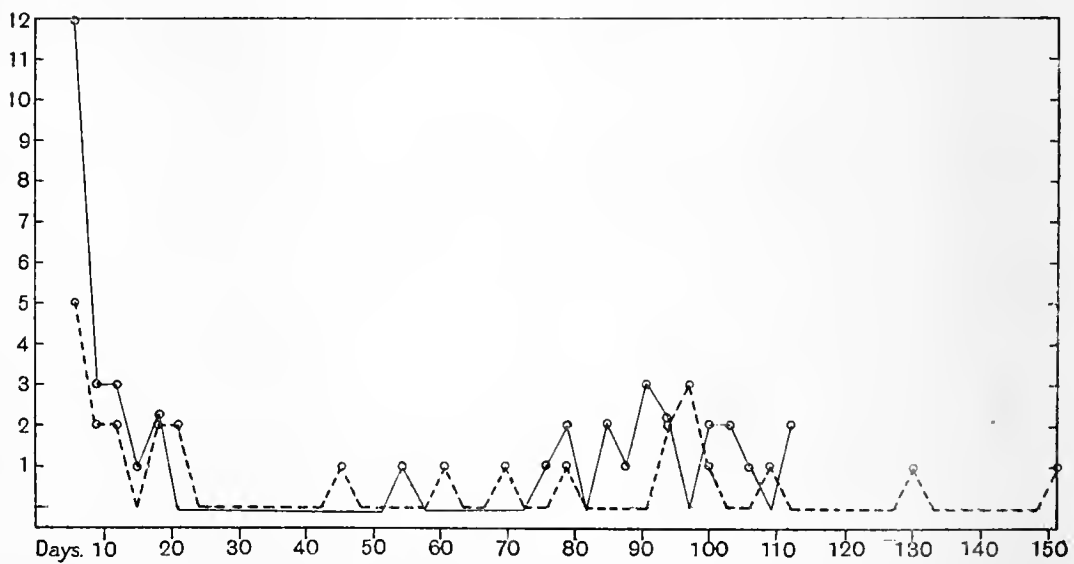


Chart 8. Emergence of *C. fasciatus* from cocoons in Incubators 85 Dry and 85 Wet (see Tables as Chart 7).

Continuous line, 85 Wet. Temperature 84° F. Humidity average .70 to .76.

Dotted line, 85 Dry. Temperature 84° F. Humidity average .60 to .63.

Vertical numbers = units of emergence. Horizontal numbers = time in days (3 day units).

on this point for this series, we may note the fact that the individuals reared under cool conditions in the cellar (7th October, 1910, Table XXVIII) during the autumn of 1910, assumed the cocoon stage during mid-winter, did not emerge during the spring but during the late summer and autumn of the following year. That is to say, that a proportion of them, after being reared under cool humid conditions, resisted the summer range of temperature with a mean average of above 60° F. for three months, and commenced their adult life on a falling temperature during the autumn of 1911.

A similar occurrence is also apparent in the Tables dealing with the cocoons spun in incubator 75 Wet, and then distributed to the cellar,

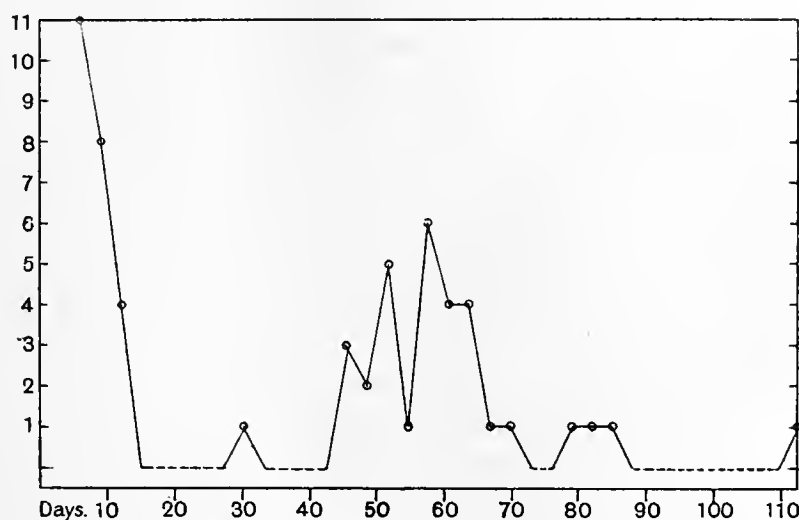


Chart 9. Emergence of *C. fasciatus* from cocoons in Incubator 75 Wet (see Exps. Jan., Feb., March 1911, Table XXXI, Nov. 1910, Table XXXII).

Temperature 74.6 to 75.5 F.

Humidity average from .76 to .84.

Vertical numbers=units of emergence. Horizontal numbers=time in days (3 day units).

laboratory cupboard and beehive (Table XXXI and Charts 10 to 12), and in the "complex transference" series, under date 8th November, 1910 (Table XXXII) spun in incubator 85 Wet, and then transferred to the cellar. No emergences at all took place between the 68th and 236th day after the cocoons were placed in the cellar. Perhaps the most striking example is afforded by an experiment in which cocoons were transferred from incubator 75 Wet to the beehive on the 23rd March, 1911 (see Table XXXI (b)). When the cocoons were put in, the mean temperature was only 42.9° F., yet some of the individuals resisted the high summer temperature of 1911, which reached a maximum average of 80° F. for the months of July and August, and emerged at much lower temperatures during the autumn.

Drought would appear to lengthen the average resting period in the cocoon at 85° F. and 92° F., and to shorten it at the lower temperature of 75° F. While there are some contradictions to be found in the tables, they do not counterbalance the general conclusion, and the massed evidence of the monthly series of tests—to be referred to immediately—is of the same tenor. The differences are not large, the duration of the cocoon period being on an average 25·3 days in 75 Wet, as against 23·8 days in 75 Dry, while at the higher

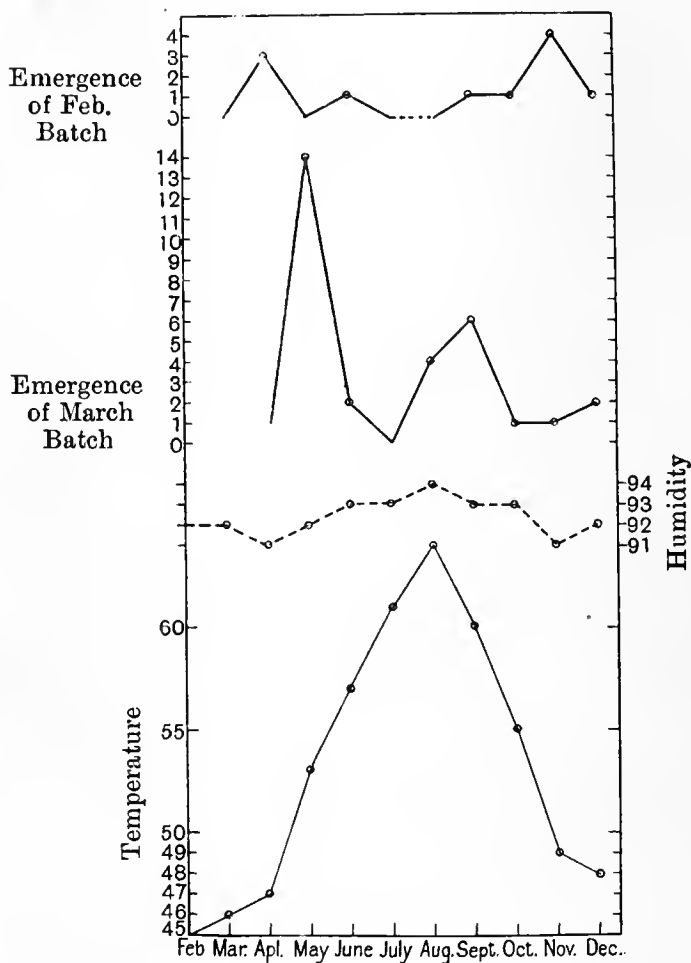


Chart 10. Influence of Temperature and Humidity upon the emergence of *C. fasciatus* from cocoons. CELLAR (see Exps. Feb. and March 1911, Table XXXI).

Two batches: one put in Feb., one put in March 1911.

Vertical numbers at top of chart give units of emergence (two series).

„ „ on Left, mean Temp. F°.

„ „ on Right, Humidity.

Horizontal divisions, time (monthly units).

*Explanation.* The two curves at the top of the diagram express the numbers of fleas emerging at monthly intervals of time. The dotted line represents the variation in humidity and the lower continuous line the variation of temperature during the period of experiment.

temperatures it is only 21·6 days in 85/93 Wet and 32·7 days in 85/93 Dry.

A very extensive series of experiments, of which only the summaries are included in Table XXXIII, were undertaken in order to investigate the existence of a seasonal fluctuation in the constitution of *C. fasciatus* which might influence the length of the resting period apart from the temperature. Full grown larvae were taken from the cages in large

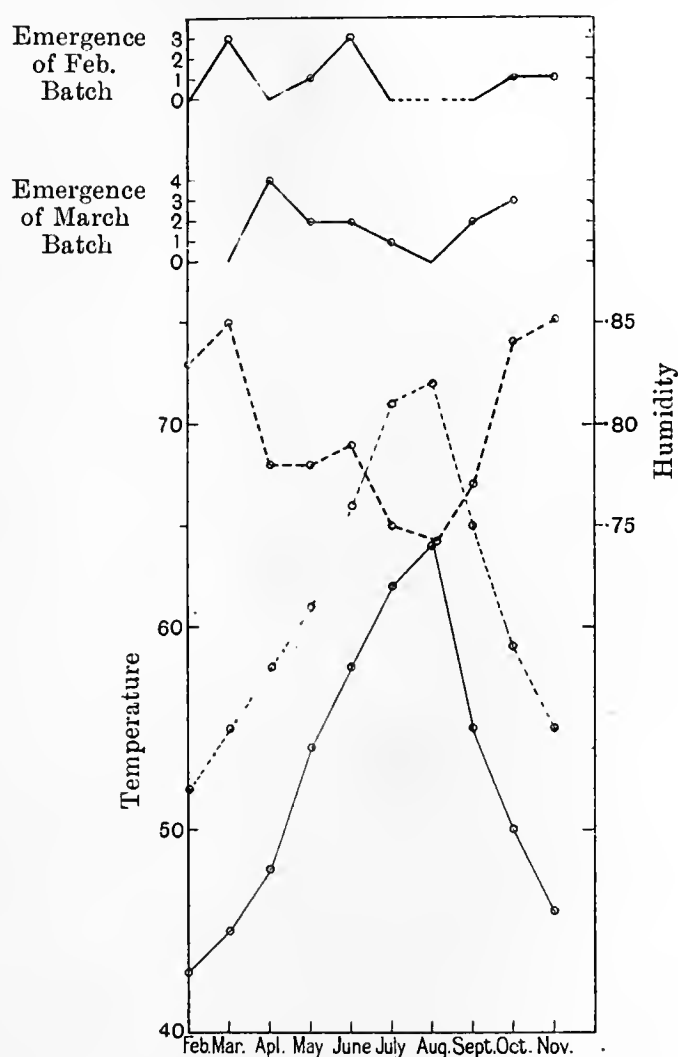


Chart 11. Influence of Temperature and Humidity upon the emergence of *C. fasciatus* from cocoons. LABORATORY CUPBOARD (see Exps. Feb. and March 1911, Table XXXI).

Two batches put in Feb. and March 1911.

Vertical numbers at top of chart give units of emergence (two series).

„ „ on Left, Temp. F°.

„ „ on Right, Humidity.

Horizontal divisions, time (monthly units).

Explanation as for Chart 10, except that temperature is represented by two curves (maxima and minima).

batches each month and placed in incubator 75° Wet, so that all the cocoons might be spun under conditions as nearly similar as possible. The cocoons were then divided into batches and distributed among the different incubators, cupboards, etc. as far as possible on identical dates.

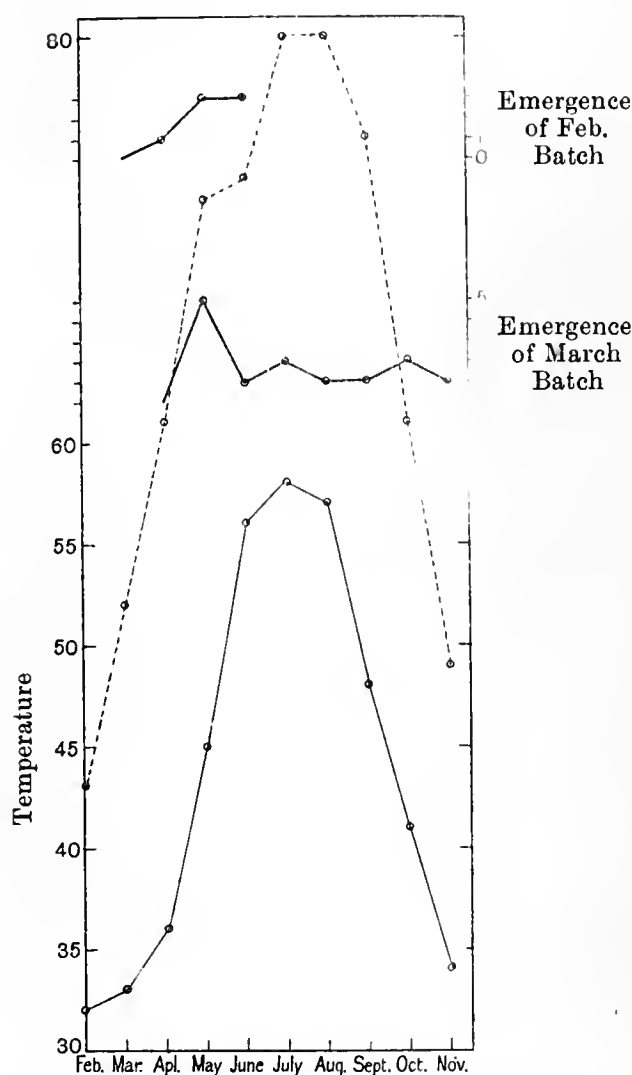


Chart 12. Influence of Temperature on emergence of *C. fasciatus* from cocoons (see Exps. Feb. and March 1911, Table XXXI).

Record of two batches in Beehive.

Vertical numbers on Right, units of emergence (two series). On Left, Temp. F.° scale. Horizontal divisions, time (monthly unit).

Explanation as for Chart 11.

Both in mortality and in tendency to "rest," there is no very clear indication of a seasonal change. The November cocoons certainly showed very definite intolerance of heat and drought. In the cool situations on the other hand there was a very marked tendency for them

to have prolonged resting periods, the average length of the cocoon period for this month being easily ahead of the others. The cocoons were also for the most part very hard.

Although the question cannot be regarded as settled, the results of the November series appear to negative the suggestion made on p. 539 that individuals which achieve lengthy periods of rest in response to cold were equally fitted for rest under hot conditions.

In short, the cocoon period of *C. fasciatus* is constitutionally a very varied one. It is possibly more correct to regard the species as having a certain percentage of individuals adapted for long resting periods in the face of extreme conditions of temperature, but susceptible to comparatively quick development in response to "warm" conditions (70° F.). There is also evidence which suggests that a "spring-autumn" emergence is favoured; individuals which have started to rest in response to low temperature resisting the early summer rise, continuing their rest in spite of a rising temperature and emerging during the autumn. It is even within the bounds of possibility that a second winter might be passed as larvae resting within the cocoons, since I have several instances of larvae resting for periods of over a year.

That a larger proportion of individuals achieve a lengthy rest under cool than hot conditions is perhaps a racial question; were we to experiment with a race of *C. fasciatus* which came from a warmer country than England, it is possible that the position might be reversed.

An experiment dealing with retarded emergence (Table XLIII) was made in the early spring of 1911. A large number of adult *C. fasciatus* were required to stock a new cage, and some 400 to 500 full-grown larvae were, therefore, taken from the cages, put into card jars with sand and shreds of B.S. rag and placed in incubators 75 Wet and 85 Wet. The fleas that emerged during March and April were used for the above purpose and did not form the subject of any special notice but the jars were examined every two or three days during May and onwards for "laggards."

The results are set forth in Table XLIII; this does not specifically belong to either the cocoon or larval series, it being uncertain when the cocoon period actually commenced. It is, however, probable that the greater portion of the time between the removal of the larvae from the cages and their emergence as fleas was passed within their cocoons. The Table is therefore included in this section and, as the numbers dealt with are considerable, they afford valuable testimony in support of the experiments, showing what a long period (up to 150 days) may

elapse, even under the influence of warm to hot conditions, before the full development of the adult is attained.

As regards the first batch, about 120 larvae were added to a jar contained in incubator 75 Wet on the 27th January, 1911; on the 4th March a further batch of larvae were added to this jar. The number of days stated are counted from the 4th March, although it is not improbable that some of the later records really belong to the January batch of larvae.

The cage referred to on p. 545 was stocked with some 200 to 250 fleas which may be termed the "forwards" of these batches. I am informed by Dr Boycott, in whose charge the cage remained for some two or three months, that three successive broods were observed in the cage with distinct intervals between them, during which the flea population fell to a minimum; very few individuals were seen in the intervals, but during the waves of emergence the cage swarmed with fleas.

*X. cheopis* (Tables XXXIV and XXXV). The effects of temperature are clear and unmistakable, the lengthening of the cocoon period being very marked at temperatures below 65° F.; situations with a mean temperature below this level as a rule give rise to a long rest within the cocoon. 65° F. would seem for this species to be equivalent to about 50° F. for *P. irritans* and 40 to 45° F. for *C. fasciatus*.

In the June 1911 series of experiments set forth in Table XXXV, the average duration of the cocoon stage in the incubators (15.5 days) is shorter than it was in the September experiments (21 days). As the other conditions in the two series were approximately the same, this difference suggests that some predisposing cause acted on the larvae before they were taken from the cages for the September experiment. Such an influence is probably not far to seek. Towards the end of September the temperature in the cages would be lower than during June, and it is also possible that the fall of temperature experienced by the larvae between the months of August and September may have provided the necessary stimulus for a longer resting period in the cocoon stage.

The degree of humidity does not seem to have had any marked influence on the length of rest in the cocoon. In the June series, there appears to be a slight advantage in the greater humidity of incubators, 85 Wet and 75 Wet, resulting in a shorter average length of cocoon period; this is, however, reversed in the September experiments when the incubators, 85 Dry and 75 Dry, give the shorter average. Corresponding contradictions are obtained when similar comparisons are made between other experiments in Table XXXV.



The results obtained are antagonistic to any supposition of an aestivating habit in the stock of *X. cheopis* used for these experiments.

In the September experiments of Table XXXV made in incubator 75 Wet, periods of 71 and 94 days in the cocoon were recorded. In the laboratory cupboard and cellar during the summer still longer times were taken for development. This must, I think, be regarded as a rest in response to moderate and cool conditions. This species is naturally adapted to a hot rather than a warm climate: a fact that is fully borne out by the susceptibility to cold or even cool conditions shown throughout these experiments.

At the high temperature of 93° F. the mortality of this species is not by any means prohibitive, provided the humidity is sufficient. Comparison of the results in the Wet and Dry incubators given under May, 1912, show this quite well (see Table XXXV).

The facts are, however, rather puzzling, with 93 Dry. They are more so in regard to 75 Dry, as the lowest readings of humidity were not by any means always accompanied by the highest mortality, and it is clear that there must be some disturbing factor. This may be due to the varied amount of ventilation that it is necessary to give to the incubators in order to keep the humidity as steady as possible. In December, where low figures for mortality were recorded, the ventilators were kept shut and all draught avoided. I fancy that the results are also affected by variation in the larvae, both in the matter of general vitality, which causes them to spin stronger or slighter cocoons (the flimsy ones being ill fitted to resist desiccation) and also in regard to the stage of preparation they have reached for the approaching metamorphosis before the cocoons are transferred to the dry incubators.

These results can only be taken to apply exactly for the race of English *X. cheopis* that were experimented with. It is quite possible that other races may possess greater powers of resistance to both heat and drought.

*Ct. canis.* The four Tables (XL (a), (b), (c) and (d)) dealing with this flea are not strictly comparable with each other. In the case of the earliest series under date 17th November 1910 (Table (b)) the figures recorded are definite as to dates of emergence but not as to the time of spinning; consequently the number of days calculated as the cocoon period are only approximate. With the July series (Table (c)), however, both series of dates are correct, but the number of cocoons used is approximate only; it was not possible to count them owing to their being spun together in masses. The experiment was the outcome of a chance

opportunity and we were too busily employed with definitely planned work to spare the time necessary to separate and count some 1300 cocoons used for this and the submergence trials. The figures given in the two April experiments (Table (a)) are also only approximate and may almost as correctly be supposed to deal with the larval as with the cocoon stage. The cocoons were left to emerge where the larvae span, with the consequence that there were emergences from cocoons that were not found. The experiment serves, however, to illustrate the effect of different conditions corresponding to incubator 75 Wet and the cellar in modifying the time of emergence, as both batches of larvae were placed under the test conditions on the same date.

It is to be noted that all the *Ct. canis* experiments are from stock naturally reared, not obtained from eggs laid under controlled conditions, nor, as with the rat fleas, bred from adults kept under exceptional, if favourable conditions. Of the November series (Table XL (b)) it may be remarked that the cellar experiment gives an approximately natural result; probably under out-of-door conditions in a kennel the emergence would have been some three weeks later. It is likely that the April series of larvae (Table XL (a)) are the progeny of adults that emerged in the spring about March, and the July larvae (Table XL (c) and (d)) follow from the adults reared from the April larvae. Such a basis suggests that *Ct. canis* produces about four or five broods a year according to the season, but it is probable that there would be overlapping of the fleas from each brood and a general emergence in August, provided warm weather prevailed. Some of the fleas emerging in autumn would probably survive the winter, but there would be few, if any, late autumn emergences under out-of-door conditions. The approximate unanimity displayed by this species in the incubators as regards the date of emergence of different individuals in one batch of cocoons (Table XL (c)) marks it off distinctly from the rat and human fleas and it seems not unlikely that the plagues of fleas said to occur suddenly, especially during late summer, are of this species.

Like *X. cheopis*, I can see no evidence of an aestivating habit from the recorded results in Table XL. A slender tail of laggards to each of the several batches makes considerably more show when the conditions are cool and moderate. Quite possibly many of these laggards are destroyed if high temperatures prevail.

Unfortunately no mortality figures can be given, as the cocoons were spun in masses, and at the period when this material was available there was no time to separate and count the cocoons accurately.

*C. gallinae* (Table XLI). The numbers available were too small to allow any but initiatory trials to be made and nothing very definite can be based on the results obtained. The chief items of interest would seem to be the low mortality in incubator 75 Dry, the frequent emergence of larvae from their cocoons followed by successful pupation and the fact that the mortality is so largely in the pupal stage. There is no evidence suggesting that this species may have an extended larval resting period within the cocoon.

*L. musculi* (Table XLII). A preliminary experiment, like that with *C. gallinae*, was undertaken; it is of some interest, but hardly affords basis for any final conclusions. This flea seems to be well adapted for the cool to moderate summer conditions of the cupboards, cellar and beehive, but not able to adjust itself readily to the high temperature of 93° F., although there is some evidence of its following *C. fasciatus* in attempting to meet unfavourable conditions both of heat and cold by resting. The recorded periods of rest are not comparable with those of *C. fasciatus*—this must be put down to want of opportunity, as it is quite uncertain how long the few examples that are now resting may take before emergence.

*L. musculi* would seem to be quite well adapted for conditions of low humidity in the cocoon stage—a fact that is significant, if we consider the habits and surroundings of its hosts in comparison with other flea hosts.

TABLE XXVIII. *Cocoons C. fasciatus*. "Continuous" series, reared from eggs, each batch maintained during egg, larval, and cocoon stage in the same situation.

Date of commencement of experiment in the egg (or larval) stage, see note below *	No. of cocoons found	Temperature	Humidity (No. of cocoons)	Incubator 75 Wet			Remarks
				Approximate duration of cocoon stage †	Average number of days in cocoon	Mortality in cocoon stage	
24 Sept. '10	7	74.4	.74	3 4 14	7 11	Nil	—
16 Oct. '10	1	74.1	.73	1 12	12	Nil	—
30 "	8	74.2	.73	4 9 10	10	Nil	—
" "	13	74.2	.73	2 10 11 14	10	Nil	—
21 Dec. '10	8	74.6	.72	3 8 9 10 11 14	16	12 %	—
14 Mar. '11	10	75.0	.79	1 6 11 13 18	11	?	5 cocoons opened 11 Aug. '11 found to be empty, probably false cocoons
24 April '11	3	75.5	.88	3 15	15	Nil	—

23 May '11	20	76.0	.86	5	9	13	Nil	Flea faeces	—
				8	12				
				6	14				
				1	44				
" "	7	76.0	.86	—	—	—	?	Rat faeces	Cocoons opened 23 Aug. '11 all empty
" "	21	75.7	.85	6	9	12	5%	B.S. Rag	1 cocoon opened contained dried up larva
				7	12				
				7	14				
Incubator 85 Wet									
22 Sept. '10	5	84.3	.72	No records, all emerged		No records	Nil	B.S. Rag	—
15 Mar. '11	6	84.3	.71	1	4	7	Nil	Rat faeces chopped fine	—
				3	7				
				1	9				
				1	12				
8 July '11	29	Aug. 84.7 Sept. 84.8 Oct. 84.8	.81 .78 .78	3	24	44	20%	B.S. Rag	Remaining cocoons opened 1 Dec. '11 contained 1 dried up flea, 5 dried up larvae, 1 empty cocoon
				6	25				
				3	32				
				2	43				
				2	47				
				1	69				
				1	71				
				3	75				
				1	103				
8 "	No cocoons		—	—	—	—	—	Rat faeces	—
13 "	6	84.9	.80	1	36	40	Nil	Flea faeces	Remaining cocoon opened and found empty, false cocoon
				1	40				
				2	40				
				1	43				

\* NOTE. Experiments in Table XXVIII (up to 14 Feb. 1911) are in direct continuity with those dealing with the egg stage given in Table II and (up to 25 June 1911) with larval stage experiments given in Table XVI.

† NOTE. It was not possible to fix the exact date of spinning; the days in cocoon (reckoned from the finding of the cocoons) are likely to be too few but their relation to each other should be correct.

TABLE XXVIII.—Continued.

Laboratory Cupboard									
Date of commencement of experiment in the egg (or larval) stage	Number of cocoons found	Temperature	Humidity	(No. of cocoons)	Approximate duration of cocoon stage Days	Average number of days in cocoon	Mortality in cocoon stage	Food during larval period	Remarks
6 Aug. '10	1	60 (approximate only to 8 Nov.) Nov. 54.0 42.8 Dec. 56.5 47.8	.84 .84 .85	1	102	—	—	B.S. Rag	Cocoon opened 1 Jan. '11 and found to contain a living larva
12 "	4	60 (approximate only to 8 Nov.)	.84	1 1 1	23 25 28	26	Nil	"	—
31 July '10	1	max. min 58.0 57.0 about .93		1	27	—	Nil	"	—
4 Aug. '10	3	58.0 57.0 about .93		1 2	21 29	26	Nil	"	—
14 "	1	58.0 57.0 about .93		1	30	—	Nil	"	—
7 Oct. '10	12 2 30/12/10 1911 1 2/ 1/11 Jan. 46.5 45.3 6 9/ 1/11 Feb. 46.4 44.8 1 19/ 1/11 Mar. 46.5 45.3 2 28/ 1/11 April 48.5 46.6 May 53.9 52.6 June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5 Oct. 56.3 54.8	Dec. 50.0 48.8 Jan. 46.5 45.3 Feb. 46.4 44.8 Mar. 46.5 45.3 April 48.5 46.6 May 53.9 52.6 June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5 Oct. 56.3 54.8	.93 .92 .92 .91 .91 .92 .93 .93 .94 .93 .92	2 1 2 2 2 1	203 219 224 269 285	236 (cocoon opened 21 Oct. '11 contained living flea)	—	"	1 opened 8 May '11 and found to contain a resting larva. Larva placed in small tube in 75 Wet, it died without undergoing change to pupa

4 April '11	3	max. min. Nov. 50·7 48·9 Dec. 48·7 47·4 1912 Jan. 47·2 45·9 Feb. 45·8 44·6	·91 ·92 ·92 ·92	—	—	—	—	Rat faeces	2 fleas emerged, 1 on 12 June, 1 on 27 July, but the cocoons they came from were not found
18 June '11	32 21 July	see above	—	2	19	71	—	Flea faeces	1 cocoon opened contained dried up pupa
				3	26				
				3	31				
				4	39				
				3	41				
				2	47				
				2	54				
				1	61				
				1	70				
				1	81				
				1	97				
				1	105				
				2	121				
				1	131				
				1	138				
				1	194				
				2	207				
25 "	27 21 July	see above	—	1	13	27	—	B.S. Rag	1 cocoon false, neither dried remains nor cast skin
				4	19				
				9	26				
				4	25				
				6	31				
				1	41				
				1	54				

TABLE XXIX. *Cocoons C. fasciatus*. "Continuous" series reared from eggs.

Date of commencement of experiment in the egg (or larval) stage, see note below†	Number of cocoons found	Temperature	Moistened	Incubator 75° Wet (moistened during the larval period)*			Mortality in cocoon stage	Food during larval period	Remarks
				(No. of cocoons)	Approximate duration of cocoon stage: Days	Average number of days in cocoon			
13 Jan. '11	9	73.7	Water	2	13	19	11%	B.S. Rag	1 cocoon opened and found to contain a dead flea
				3	14				
				1	26				
				1	27				
				1	36				
28 Dec. '10	6	74.7	Urine	2	11	11	50%	"	3 cocoons opened and dead fleas found in them
				1	10				
"	14	74.6	Water	2	13	16	64%	"	9 cocoons opened contained 8 dead larvae, 1 dead flea
				2	18				
				1	21				
4 Mar. '11	2	74.6	Urine	2	13	13	Nil	"	only 2 cocoons found
"	1	74.6	Water	1	10	10	Nil	"	—
31 Jan. '11	3	75.7	Water daily	1	10-13	—	67%	"	2 cocoons opened contained dead larvae
"	No cocoons	—	Urine daily	—	—	—	—	"	—
30 Dec. '10	7	85.3	Urine at intervals	5	13	—	29%	"	2 cocoons opened contained dried up pupae
"	No cocoons	—	Water at intervals	—	—	—	—	"	—
24 Feb. '11	2	83.5	Urine 1 c.c. daily	1	3	5	Nil	"	—
				1	6			"	—
"	4	84.2	Water 1 c.c. daily	1	16	18	50%	"	2 cocoons opened contained dead larvae
				1	20				





TABLE XXX. *Cocoons C. fasciatus*. "Continuous" series, contrasted experiments (compiled from Table XXVIII); larvae from same batch of eggs.

Date of commencement of experiment in the larval stage	(a) Reared on the same food under different conditions of Temperature and Humidity.									
	Place Reared from egg in Incubator 75 Wet	Food Rat faeces crushed	Number of cocoons found	Temp 75.0	Humidity .79	Approximate duration of cocoon stage (No. of cocoons)	Average number of days in cocoon	Mortality in cocoon stage Doubtful if any		
14 March '11			10			1	6	11		
						1	10			
						2	12			
						1	17			
						5 cocoons opened 11 August '11 and found empty, probably false cocoons				
15 March '11	Incubator 85 Wet	"	6	84.3	.71	1	4	7		Nil
						3	7			
						1	9			
						1	12			
Date of commencement of experiment in the egg stage	(b) Reared under the same conditions of Temperature and Humidity but on different food.									
		Reared from hatching on B.S. Rag	13	74.2	.73	3	8	10		"
30 Oct. '10	Incubator 75 Wet					1	9			
						6	10			
						2	11			
						1	14			
"	"	Rat faeces	8	74.2	.73	4	9	10		"
						2	10			
						1	11			
						1	14			

TABLE XXXI (a). Cocoons *C. fasciatus*. Full grown larvae taken from the breeding cages and allowed to spin in Incubator 75 Wet (Temperature 74° F., Humidity 70); cocoons then placed in different situations.

Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temperature	Humidity	Dates of emergence of fleas	No. of days in cocoon	Average days	Remarks
30 Jan. '11	15	Incubator 85 Wet	84.0	.70	4 7 Feb. '11 1 10 " 2 13 " 1 20 " 1 16 April '11 1 21 " 1 12 May '11	8 11 14 21 76 81 102	32	23 March '11. Examined, 5 "soft" all empty, 3 "hard" contained resting larvae 7 June '11. 5 "hard" and 1 "soft" opened, 1 contained living resting larva, remainder contained either dried up larvae or cast larval skins. Mortality doubtful
"	15	Incubator 85 Dry	84.1	.60	2 7 Feb. '11 2 8 " 1 20 " 1 23 " 1 24 " 1 6 April '11 1 27 " 1 3 July '11	8 9 21 24 25 66 87 154	41	19 Feb. '11. 7 empty cocoons opened all "soft" or tending in that direction, but not flimsy cocoons, the silk forming a continuous papery lining. One very little if at all stronger contained a resting larva. 7 "hard" cocoons unopened. Mortality doubtful 7 June '11. One of the hard cocoons opened found to contain a living pupa, the adult flea within the pupal envelope being nearly ready to emerge. Mortality doubtful
"	15	Incubator 75 Wet	75.5	.76	3 7 Feb. '11 1 10 " 1 21 March '11 2 24 " 1 2 April '11 1 3 "	8 11 50 53 62 63	35	23 March '11. 6 "soft" cocoons opened, 4 empty, 1 contained pupa with flea nearly ready to emerge, 1 a dead flea. 2 "hard" cocoons contained "white," that is recently developed, pupae 7 June '11. Remainder of cocoons opened contained either dried up larvae or cast larval skins. Mortality doubtful
"	15	Incubator 75 Dry	75.6	.49	3 7 Feb. '11 1 8 " 2 13 " 1 14 " 1 17 " 1 18 March '11 *1 25 " 1 27 " 1 31 "	8 9 14 15 18 47 54 56 60	26	13 March '11. 1 hard cocoon opened contained a resting larva which successfully pupated and an adult flea was reared on the 25th March, see column 6* Note the silk of this cocoon was hard and brown forming an almost horny lining to the cocoon June '11. Remaining cocoons opened contained dead larvae. Mortality 20 1/2%



TABLE XXXI (b). *Cocoons C. fasciatus. Full grown larvae taken from the breeding cages and allowed to spin in Incubator 75 Wet (Temperature 74.3° F., Humidity 76); cocoons then placed in different situations.*

Special experiment with cocoons from one batch of larvae taken from cages on the same date, 24 March 1911.									
Date on which the cocoons were placed under test conditions	Number of cocoons	Place	Temperature	Humidity	Dates of emergence of fleas	No. of days in cocoon	Average	Mortality	Remarks
24 March '11	15	Incubator 85 Wet	84.0	.76	5 2 April '11 2 3 " " 1 4 " " 1 6 " " 1 13 June '11 1 4 July '11	9 10 11 13 81 102	24	27%	Remaining cocoons opened 2 Oct. 1911 and found to contain dried up larvae
23 March '11	15	Incubator 85 Dry	84.0	.60	1 31 March '11 1 2 April '11 1 3 " " 1 7 " " 1 8 " " 1 15 " " 1 19 " " 1 9 June '11 3 5 July '11	9 11 12 15 16 23 27 78 104	46	27%	Remaining cocoons opened 23 Oct. 1911 contained 3 dried up fleas, 1 dried up larva
24 March '11	15	Incubator 75 Wet	75.4	.84	2 3 April '11 1 14 May '11 1 22 " " 1 23 " " 1 24 " " 1 30 " " 1 14 June '11 1 15 " " 1 19 " " 1 17 July '11	10 53 59 60 61 67 82 83 87 115	62	27%	Remaining cocoons opened 2 Oct. 1911 contained dried up larvae
"	15	Incubator 75 Dry (II)	75.7	.47	2 3 April '11 1 6 " "	10 13	11	80%	Remaining cocoons opened 28 Sept. 1911 contained dried up larvae
"	15	Incubator 75 Dry (I)	75.7	.47	2 3 " " 1 4 " " 1 6 " "	10 11 13	11	76%	Remaining cocoons opened 28 Sept. 1911 contained dried up larvae
"	15	Lab. cupboard	max. min. March 55.2 45.5 April 58.2 48.2 May 61.8 54.3 June 66.4 58.2 July 71.5 62.7 Aug. 72.0 64.1 Sept. 65.7 55.7 Oct. 59.0 50.9	.85 .78 .78 .79 .75 .74 .77 .84	4 29 " " 2 9 May '11 1 17 June '11 1 23 " " 1 27 July '11 1 20 Sept. '11 1 22 " " 1 9 Oct. '11 1 13 " " 1 23 " "	36 46 85 91 125 180 182 199 203 213	108	7%	1 cocoon opened contained dried up larva

TABLE XXXI (b)—Continued.

Date on which the cocoons were placed under test conditions	Number of cocoons	Place	Temperature max. min.	Humidity	Date of emergence of fleas	No. of days in cocoon	Average	Mortality	Remarks
24 March '11	15	Cellar I	March 46.0 44.7 April 48.5 46.6 May 53.9 52.6 June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5 Oct. 56.3 54.8 Nov. 50.7 48.9	.93 .91 .92 .93 .93 .94 .93 .92 .91	2 13 May '11 2 18 " 3 27 " 1 3 June '11 1 26 " 2 9 Aug. '11 2 21 " 1 14 Sept. '11 1 26 " 1 13 May '11 4 18 " 2 27 " 1 11 Sept. '11 1 19 " 2 26 " 1 16 Oct. '11 1 15 Nov. '11 1 7 Dec. '11 1 25 " 1 9 May '11 2 13 " 1 18 " 1 30 " 1 14 June '11 1 14 July '11 1 24 " 1 2 Aug. '11 1 25 Sept. '11 1 30 " 1 3 Oct. 1 27 " 1 4 Nov.	50 57 64 70 93 138 150 174 186 50 57 64 171 179 186 206 236 258 276 47 51 58 68 83 113 123 132 186 191 194 218 226	100	Nil	—
"	15	Cellar II	(as above)				140	Nil	—
23 March '11	15	Beehive	March 52.5 33.3 April 61.7 36.8 May 72.8 45.2 June 73.0 56.5 July 80.3 58.6 Aug. 80.2 57.6 Sept. 75.3 48.0	— — — — — —			124	6%	1 cocoon opened 30 Sept. '11 contained living flea Remaining cocoons contained dead flea
24 March '11	15	Warm cupboard	Dry March 61.7 April 63.0 May 62.9*	.58 .57 .60	3 7 April '11 2 9 " 1 10 " 1 11 " 1 15 " 1 17 " 2 23 May '11 1 24 " 1 30 "	14 16 17 18 22 24 60 61 67	31	Nil	Remaining cocoons opened 30 Sept. '11 empty. ? "false" cocoon

TABLE XXXI (c). *Cocoons C. fasciatus. Full grown larvae taken from the breeding cages and allowed to spin in Incubator 75 Wet (Temperature 73° F., Humidity 70); cocoons also maintained in Incubator 75 Wet.*

Contrast between cocoons kept in card jar and tube.									
Date on which the cocoons were placed under test conditions	Number of cocoons	Receptacle	Temperature	Humidity	Dates of emergence of fleas	Number of days in cocoon	Average	Mortality	Remarks
20 Feb. '11	15	Tube	74.6	.77	1 1 March '11	9	37	27 %	Remainder of cocoons opened 17 Aug. '11, found 1 dried up pupa, 3 dried up larvae
					2 2    "	10			
					2 4    "	12			
					2 13 April '11	52			
					1 15    "	54			
					1 21    "	60			
					1 24    "	63			
					1 2 May '11	71			
"	15	Jar	74.6	.77	2 28 Feb. '11	8	41	20 %	Remainder of cocoons opened 18 Aug. '11, found 1 dried up flea, 2 dried up larvae
					1 1 March '11	9			
					1 4    "	12			
					3 10 April '11	49			
					1 15    "	54			
					1 20    "	59			
					1 21    "	60			
					1 24    "	63			
					1 30    "	69			

TABLE XXXII. *Cocoons C. fasciatus. Full grown larvae taken from breeding cages and sorted into batches; these allowed to spin under one set of conditions and transferred to contrasting conditions for the cocoon stage.*

Date on which the larvae were taken from the breeding cages	No. of larvae	Place in which the larvae formed their cocoons and dates on which they were found and transferred	Temp. max. min.	Humidity	Place to which the cocoons were transferred and dates of emergence of fleas	Temp.	Humidity	Estimated no. of days in cocoon	Average	Mortality
11 Nov. '10	18	Cellar 2 16 Nov. '10 1 18 " 3 22 " 4 25 " 2 28 " 5 1 Dec. '10	47.6 46.2	.92	Incubator 85 Wet* 1 25 Nov. '10 3 6 Dec. '10 1 16 Jan. '11 1 15 Feb. '11 1 20 " 1 23 " 1 26 " 2 28 " 1 3 " 2 16 " 1 17 " 2 25 "	84.1	.70	1 took 9 days 1 " 14 1 " 18 1 " 20 1 " 55 1 " 85 1 " 87 1 " 90 2 " 92 1 " 93 1 " 94 1 " 95 2 " 105 1 " 106 2 " 114	77	Nil
8 Nov. '10	13	Incubator 85 Wet 9 9 Nov. '10 4 11 "	84.1	.70	Cellar 5 30 Dec. '10 1 2 Jan. '11 1 8 " 1 9 " 1 12 " 1 19 " 1 5 July '11 1 25 Sept. '11† 1 5 Oct. '11	max. min. Nov. 48.6 47.1 Dec. 50.9 48.8 Jan. 46.5 45.3 Feb. 46.4 44.8 Mar. 46.5 45.3 Apr. 48.5 46.6 May 53.9 52.6 June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5	.92 .93 .92 .92 .91 .91 .92 .93 .93 .94 .93	5 took 51 1 " 53 1 " 59 1 " 60 1 " 63 1 " 68 1 " 236 1 " 319 1 " 328	111	Nil
6 Nov. '10	19	Laboratory cupboard 3 7 Nov. '10 1 8 " 2 11 " 4 13 " 3 15 " 3 17 " 2 23 " 1 28 "	54.0 42.8	.84	Incubator 75 Wet 3 18 Nov. '10 2 22 " 1 25 " 2 27 " 1 17 Dec. '10 1 9 Jan. '11 3 21 "	74.3	.75	4 took 11 4 " 14 1 " 34 1 " 57 3 " 67	30	Doubtful
28 Oct. '10	18	Incubator 75 Wet 18 2 Nov. '10	73.9	.74	(6 cocoons opened 7 June '11 were empty in some cases, in others contained dried up larvae) Laboratory cupboard 3 28 Nov. '10 7 6 Dec. '10 3 8 " 1 15 " 2 19 "	55.5 46.4	.85	3 took 26 7 " 34 3 " 36 1 " 43 2 " 47	33	6 0/0

\* 18 fleas emerged from 17 cocoons, the inference is that two cocoons were spun together.

† Cocoon opened, living flea emerged.



11 Apr. '11	26	Cellar 1 13 April '11 3 17 " 9 22 " 3 25 " 1 27 " 2 23 May '11 5 27 " 1 8 June '11 1 26 "	55.1 53.5	.92	Incubator 85 Dry 1 4 May '11 1 6 June '11 1 26 July '11 2 2 Aug. '11 1 17 " 1 4 Sept. '11	84.0	.63	1 took 21 1 " 50 1 " 100 1 " 102 1 " 107 1 " 117 1 " 135	90	73 %
					(19 cocoons opened 23 Oct. '11 contained 1 dried up flea, 1 dried up pupa, 17 dried up larvae)					
	26	Incubator 85 Dry 4 13 April '11 4 15 "	84.0	.60	Cellar 1 27 May '11 1 3 June '11 1 26 " 1 27 July '11 2 1 Aug. '11 1 26 Sept. '11	max. min. Apr. 48.5 44.6 May 53.9 52.6 June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5	.91 .92 .93 .93 .94 .93	1 took 44 1 " 51 1 " 74 1 " 105 2 " 108 1 " 133	89	Nil
		(2 larvae emerged from their cocoons and one died, the remainder died without spinning)								
	26	Laboratory cupboard 3 18 April '11 2 19 " (Remainder of larvae died)	59.2 46.6	.77	Incubator 75 Dry	75.0	.50	—	—	100 %
					(Cocoons opened 28 Sept. '11 contained dried up larvae)					
	26	Incubator 75 Dry (Larvae all died without spinning)	76.0	.49	—	—	—	—	—	—
	26	Warm cupboard (Larvae all died without spinning)	62.3	.54	—	—	—	—	—	—
	26	Incubator 75 Wet 12 13 April '11 3 15 " 1 16 " 1 12 May '11 2 15 "	75.2	.80	Warm cupboard 2 26 April '11 1 2 May '11 1 29 " 1 2 June '11 (13 cocoons opened 30 Sept. '11 contained 9 dried up larvae, 1 dried up pupa, 2 empty "false" cocoons)	Apr. 63.0 May 62.9 June 66.6	.57 .60 .65	2 took 13 1 " 19 1 " 46 1 " 50	28	58 %
		(2 larvae emerged from cocoons before transference)								
	26	Incubator 85 Wet 11 12 April '11 8 13 "	84.6	.72	Beehive 7 13 May '11 4 23 " 2 26 " 2 10 June '11 2 14 " 1 24 " 1 2 Aug. '11	74.1 49.6	—	7 took 31 4 " 41 2 " 43 2 " 58 2 " 62 1 " 72 1 " 111	47	Nil
		(Remainder of larvae lost owing to accident)				(Averages)				

TABLE XXXII.—Continued. *Cocoons C. fasciatus.*

Date on which the larvae were taken from the breeding cages	No. of larvae	Place in which the cocoons were formed and dates on which they were found and transferred	Temp. max. min.	Humidity	Place to which the cocoons were transferred and dates of emergence of fleas	Temp.	Humidity	Estimated no. of days in cocoon	Av.	Mortality
11 Apr. '11	26	Beehive 1 19 April '11 (25 larvae died)	69.6 33.3	—	Incubator 85 Wet	83.0 (No emergence from cocoon)	.76	—	—	—
*3 June '11	Unre- corded	Incubator 85 Wet 22 6 June '11	76.4	.93	Cellar 3 5 July '11 1 12 " 1 27 " 1 1 Aug. '11 2 9 " 1 11 " 3 21 " 1 11 Sept. '11 1 14 " 2 19 " 1 26 " 1 16 Oct. '11 1 1 Nov. '11 1 15 " 1 20 " 1 7 Dec. '11	1911 max. min. June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5 Oct. 56.3 54.8 Nov. 50.7 48.9 Dec. 48.7 47.4 1912 Jan. 47.2 45.9 Feb. 45.8 44.6 Mar. 49.1 48.1 Apr. 50.3 49.1	.93 .93 .94 .93 .92 .91 .92  .92 .92 .93 .93	3 took 24 1 " 36 1 " 51 1 " 56 2 " 64 1 " 66 3 " 76 1 " 97 1 " 100 2 " 105 1 " 112 1 " 132 1 " 148 1 " 162 1 " 167 1 " 184	88	Nil
"	Unre- corded	Incubator 75 Wet 14 6 June '11 17 8 "	1911 June 75.8	.86	Cellar 3 5 July '11 4 12 " 3 11 Aug. '11 2 21 " 1 11 Sept. '11 3 14 " 4 19 " 1 26 " 2 5 Oct. '11 1 16 " 1 1 Nov. '11 1 9 " 1 26 April '12	(as above)		3 took 29 4 " 36 3 " 66 2 " 76 1 " 97 2 " 98 1 " 100 4 " 103 1 " 110 2 " 119 1 " 130 1 " 146 1 " 154 1 " 323	91	about 3%

(Remaining cocoons opened contained 1 dead flea, 3 false cocoons)

TABLE XXXIII. *Cocoon C. fasciatus. Monthly test. June 1911 to February 1912. Full grown larvae taken from the breeding cages and allowed to spin in Incubator 75 Wet; cocoons then removed to various situations.*

Date on which the cocoons were placed under test conditions	Number of cocoons	Incubator 75 Wet.					Number of fleas emerging	Mortality	Remarks	
		Temperature	Humidity	Min. days in cocoon	Max. days in cocoon	Average number of days in cocoon				
17 June '11	26	June 75.8	.86	10	59	16	26	Nil	—	
11 July '11	22	July 76.3	.85	13	72	42	21	Nil	1 "false" cocoon	
15 Aug. '11	22	Aug. 75.0	.80	13	59	24	21	Nil	"	
20 Sept. '11	22	Sept. 74.8	.76	12	54	27	18	10 0/0	" "false" cocoons	
20 Oct. '11	22	Oct. 74.4	.73	11	57	25	17	14 0/0	as fleas, 2 "false" cocoons	
15 Nov. '11	22	Nov. 75.0	.69	10	82	31	22	Nil	—	
10 Dec. '11	22	Dec. 75.8	.65	9	64	15	18	18 0/0	9 0/0 as larvae, 9 0/0 as fleas	
5 Jan. '12	22	1912	.71	15	58	22	21	Nil	1 "false" cocoon	
11 Feb. '12	22	Jan. 75.5	.76	8	79	27	20	5 0/0	as flea, 1 "false" cocoon	
		Feb. 75.0	.76							
		Mar. 75.1	.79							
		April 75.8	.79							
		May 75.6	.86							
		June 74.9	.83							
		July 75.0	.86							
		Aug. 74.3	.81							
Incubator 75 Dry.										
17 June '11	22	June 75.2	.53	9	27	15	20	10 0/0	as larvae	
11 July '11	22	July 76.3	.57	13	70	31	4	72 0/0	" 2 "false" cocoons	
14 Aug. '11	22	Aug. 75.7	.59	11	23	14	22	Nil	—	
18 Sept. '11	22	Sept. 74.9	.54	10	45	18	14	27 0/0	22 0/0 as larvae, 5 0/0 as fleas, 2 "false" cocoons	
20 Oct. '11	22	Oct. 74.6	.52	11	60	24	19	Nil	3 "false" cocoons	
16 Nov. '11	22	Nov. 74.4	.54	11	68	28	6	72 0/0	as larvae	
12 Dec. '11	22	Dec. 74.7	.51	12	70	24	20	9 0/0	"	
4 Jan. '12	22	1912	.51	8	128*	34	20	9 0/0	"	
11 Feb. '12	22	Jan. 75.2	.50	11	86	36	17	14 0/0	"	
		Feb. 73.5	.56							
		Mar. 73.2	.56							
		April 74.1	.56							
		May 74.7	.59							
		June 75.8	.54							
		July 76.2	.64							
		Aug. 75.0	.56							

\* A cocoon opened on the 11th May contained a living flea.

TABLE XXXIII.—Continued.

Date on which the cocoons were placed under test conditions	No. of cocoons	Temperature	Humidity	Incubator 85/93 Dry.			Mortality	Remarks
				Min. days in cocoon	Max. days in cocoon	Average number of days in cocoon		
19 June '11	28	June 83.9	.60	8	117	32	43 0/0	4 0/0 fleas, 39 0/0 larvae
19 July '11	22	July 84.3	.64	14	92	48	36 0/0	31 0/0 larvae, 5 0/0 fleas
14 Aug. '11	22	Aug. 83.8	.63	14	66	23	say 9 0/0	1 dead flea, 1 dead larva, 5 empty cocoons, doubtful if "false" cocoons or larval remains past recognition
21 Sept. '11	22	Sept. 84.4	.59	11	40	17	10 0/0	5 0/0 larvae, 5 0/0 fleas, 3 "false" cocoons
20 Oct. '11	22	Oct. 84.7	.60	18	24	21	30 0/0	10 0/0 fleas, 20 0/0 larvae, 3 "false" cocoons, 2 cocoons opened 16 March contained living but somewhat shrunken larvae which subsequently died
14 Nov. '11	22	Nov. 92.9	.56	1 flea emerged in 7 days	7	1	72 0/0	2 larvae emerged from cocoons 20 Nov. and died; cocoons opened 16 March contained 11 dead and dried larvae, 1 doubtful if dead as it still retained its natural colour and appearance but did not move, 2 dead fleas, remainder "false" cocoons
12 Dec. '11	22	Dec. 92.8	.57	10	118	77	doubtful	cocoons examined 16 March '12; 4 opened contained 3 resting larvae*, 1 empty; the remaining cocoons and 3 extracted larvae were transferred to Incubator 75 Wet and 6 fleas emerged; on 31 May 1912 a cocoon opened contained a dead flea
13 Feb. '12	22	Jan. 93.2	.56	—	—	—	100 0/0	cocoons opened 28 June contained 8 dried up larvae, 5 dried up pupae, 9 dried up fleas
19 June '11	28	Feb. 93.0	.60	Incubator 85/93 Wet.			as larvae	
19 July '11	22	Mar. 93.1	.60	8	74	19	10 0/0	as larvae
14 Aug. '11	22	Apr. 93.7	.83	12	105	42	15 0/0	5 0/0 as larvae, 10 0/0 as fleas
20 Sept. '11	22	May 93.3	.81	7	42	28	22 0/0	as pupae
20 Oct. '11	22	June 93.0	.77	7	30	13	5 0/0	5 0/0 as fleas, 5 0/0 as larvae; 2 cocoons opened 11 May '12 contained resting larvae (204 days); they were transferred to Incubator 75 Wet with 1 unopened cocoon; the resting larvae died, the cocoon was opened 12 June and found to contain a dead larva.
		July 93.0	.78	13	31	15	10 0/0	5 0/0 as fleas, 5 0/0 as pupae, 59 0/0 as larvae; 23 0/0 as fleas, 9 0/0 as pupae, 18 0/0 as larvae; 2 resting larvae taken from their cocoons after 203 days were transferred to Incubator 75 Wet but died
		Aug. 93.0	.73					
			.69					
			.66					
15 Nov. '11	22	Nov. { to 23rd 85.2 to 30th 75.0	.66	10	28	14	69 0/0	
12 Dec. '11	22	Dec. 92.4	.68	7	25	14	50 0/0	
		1912	.67					
		Jan. 92.6	.69					
		Feb. 92.4	.66					
		Mar. 92.3	.66					
		April 93.1	.66					
		May 95.1	.66					
		June 94.5	.66					

23 0/0 as fleas, 4 0/0 as pupae, 14 0/0 as larvae;  
2 of the larvae though shrunken were still  
fresh and natural in appearance as though  
but recently dead  
23 0/0 as fleas, 4 0/0 as pupae, 41 0/0 as larvae;  
3 of these larvae though shrunken were  
still fresh and natural in appearance as  
though but recently dead

41 0/0  
68 0/0

12  
4

14  
28

24  
72

12  
9

.74  
.75

July 95.2  
Aug. 94.2

22  
22

6 Jan. '12  
11 Feb. '12

Warm Cupboard.

—  
4 0/0 as fleas, 68 0/0 as larvae  
as larvae  
"  
"  
5 0/0 as fleas, 59 0/0 as larvae, 1 "false" cocoon  
as larvae, 2 "false" cocoons  
"  
"  
1 "false" cocoon

Nil  
72 0/0  
27 0/0  
5 0/0  
14 0/0  
64 0/0  
9 0/0  
18 0/0  
14 0/0

—

21  
54  
25  
33  
31  
43  
12  
26  
25

62  
68  
56  
141  
98  
91  
17  
78  
108

11  
14  
14  
14  
14  
18  
10  
14  
14

.65  
.65  
.66  
.66  
.59  
.58  
.62  
.60  
.63  
.59  
.64  
.70  
.73  
.71  
.71

June 66.6  
July 71.3  
Aug. 75.0  
Sept. 66.3  
Oct. 67.4  
Nov. 63.0  
Dec. 62.3  
1912  
Jan. 59.7  
Feb. 59.5  
Mar. 60.7  
April 59.0  
May 62.6  
June 65.0  
July 67.3  
Aug. 64.5

22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22

17 June '11  
10 July '11  
14 Aug. '11  
16 Sept. '11  
23 Oct. '11  
13 Nov. '11  
11 Dec. '11  
4 Jan. '12  
19 Feb. '12

Laboratory Cupboard.

—  
—  
as larvae  
1 larva emerged from cocoon and died; 1 dead  
flea found in cocoon; 1 resting larva taken  
from cocoon after 357 days  
as larvae; 1 resting larva taken from cocoon  
as larvae  
as fleas, died in cocoons; 1 living pupa taken  
from cocoon after 270 days  
as flea, died in cocoon; 1 living pupa taken  
from cocoon after 246 days, 1 resting larva  
taken from cocoon after 246 days  
1 living flea taken from cocoon after 198 days,  
1 living pupa taken from cocoon after 198  
days

Nil  
Nil  
9 0/0  
9 0/0  
5 0/0  
5 0/0  
9 0/0  
5 0/0  
Nil

—

42  
74  
37  
73  
64  
261  
101  
59  
67

83  
218  
73  
350  
222  
352  
257  
107  
198

23  
23  
19  
24  
32  
39  
24  
44  
36

.79  
.75  
.74  
.77  
.84  
.85  
.87  
.89  
.86  
.76  
.83  
.87  
.81  
.79  
.84

max. min.  
June 66.4 58.2  
July 71.5 62.7  
Aug. 72.0 64.1  
Sept. 65.7 55.7  
Oct. 59.0 50.9  
Nov. 54.9 46.1  
Dec. 53.9 46.2  
1912  
Jan. 52.5 44.9  
Feb. 54.4 45.3  
Mar. 56.0 47.8  
April 60.6 50.4  
May 64.0 56.8  
June 64.6 58.3  
July 68.5 62.1  
Aug. 63.3 56.3

22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22  
22

15 June '11  
10 July '11  
11 Aug. '11  
15 Sept. '11  
20 Oct. '11  
13 Nov. '11  
11 Dec. '11  
4 Jan. '12  
21 Feb. '12

\* One of the extracted larvae pupated on the 27th March '12 and the flea emerged on the 3rd April '12.  
NOTE. Mortality "as fleas" implies that death occurred in cocoons.

TABLE XXXIII.—Continued.

Date on which the cocoons were placed under test conditions	No. of cocoons	Temperature		Humidity	Min. days in cocoon	Max. days in cocoon	Average number of days in cocoon	Mortality	Remarks
		max.	min.						
17 June '11	22	73.0	56.5	—	23	97	37	Nil	4 "false" cocoons
10 July '11	22	80.3	58.6	—	14	82	37	30 0/0	25 0/0 died as larvae, 5 0/0 died as pupa, 2 "false" cocoons
11 Aug. '11	22	80.2	57.6	—	17	45	34	30 0/0	died as larvae, 2 "false" cocoons
15 Sept. '11	22	75.3	48.0	—	28	75	40	Nil	1 resting larva found on opening cocoons on 22 June '12 living after 272 days, it died without pupating
23 Oct. '11	22	61.9	41.3	—	74	319	125	Nil	1 resting larva taken from cocoon after 255 days, 1 living flea taken from cocoon after 319 days
13 Nov. '11	22	46.8	35.0	—	99	324	196	5 0/0	as flea; 1 living larva taken from cocoon after 234 days, 1 living larva taken from cocoon after 298 days, 1 living pupa taken from cocoon after 298 days, 1 living flea taken from cocoon after 298 days
11 Dec. '11	22	46.6	34.0	—	33	180	98	Nil	—
4 Jan. '12	22	43.6	34.3	—	86	246	117	23 0/0	9 0/0 larvae, 5 0/0 pupa, 9 0/0 fleas; 1 living flea taken from cocoon on 6 Sept., 1 living pupa taken from cocoon on 6 Sept.
24 Feb. '12	22	46.9	37.3	—	45	195	92	Nil	1 living larva taken from cocoon after 517 days
15 June '11	22	67.9	47.3	—	Cellar.				
10 July '11	22	67.7	49.9	—	27	109	49	Nil	—
11 Aug. '11	22	73.4	55.4	—	22	390	78	Nil	1 resting larva found in cocoon after 348 days, 1 resting larva found in cocoon after 424 days
15 Sept. '11	22	64.9	45.6	—	17	122	33	4 0/0	as flea
23 Oct. '11	22	58.1	56.7	.93	31	329	88	10 0/0	5 0/0 as larva, 5 0/0 as pupa; 1 resting larva from very hard cocoon after 281 days, 1 living pupa after 358 days
13 Nov. '11	22	61.6	60.4	.93	35	119	57	5 0/0	as larva; 1 resting larva from cocoon after 243 days, 1 living pupa from cocoon after 320 days
11 Dec. '11	22	64.4	63.4	.94	53	450	296	9 0/0	2 dead larvae, 1 living larva taken from cocoon after 222 days
4 Jan. '12	22	60.6	59.5	.93	35	303	131	5 0/0	1 dead larva, 2 living fleas taken from cocoon after 271 days
19 Feb. '12	22	56.3	54.8	.92	63	247	92	Nil	1 living flea extracted from cocoon after 247 days
	22	50.7	48.9	.91	37	245	83	Nil	2 living pupae taken from cocoons after 201 days
		48.7	47.4	.92					
		47.2	45.9	.92					
		45.8	44.6	.92					
		49.1	48.1	.93					
		50.3	49.1	.93					
		54.2	53.2	.93					
		56.4	55.6	.93					
		60.1	59.3	.93					
		57.5	56.5	.93					

NOTE. Mortality "as fleas" implies that death occurred in cocoons.

TABLE XXXIV. *Cocoons X. cheopis*. "Continuous series\*," each batch maintained during larval and cocoon stage in the same situation.

Date of com- mencement of experiment in larval stage	Number of cocoons found	Cellar.			Food	Remarks
		Temperature max. min.	Humidity	Approximate duration of cocoon stage No. of cocoons	Average number of days in cocoon	Mortality in cocoon stage
6 June '11	2	Aug. 64.5 63.4	.94	1	147	—
3 July '11	13	Sept. 60.6 59.5	.93	1	41	50 %
		Oct. 56.3 54.8	.92	3	68	30 %
		Nov. 50.7 48.9	.91	1	77	
		Dec. 48.7 47.4	.92	1	96	
				1	114	
"	14			1	191	
				3	34	
				2	41	
				1	50	
				2	91	
				1	96	
				1	114	
				1	128	
					69	14 %
11 Aug. '11	12	84.8	.78	7	11	Nil
"	7	84.8	.78	4	15	
				1	18	
				1	7	
				3	11	
				3	15	
4 Sept. '11	22	84.7	.78	1	9	Nil
				2	11	
				1	12	
				9	14	
				5	15	
				2	18	
					13	
"	21	74.7	.70	1	16	Nil
				4	17	
				7	19	
				5	20	
				2	24	
				2	46	
					22	

\* Experiments in Table XXXIV are in direct continuity with those dealing with the larval series in Table XX.

TABLE XXXV. Cocoons X. Cheopis. Full grown larvae taken from the cages and allowed to spin in Incubator 75 Wet (Temp. 76° F., Humidity .84); cocoons then placed in different situations.

NOTE. Experiments on the same date are from the same batch of cocoons.

Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temperature max. min.	Humidity	Date of emergence of fleas	Duration of cocoon stage No. of cocoons Days	Aver. days	Mortality per cent.	Remarks
16 June '11	14	Cellar	June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5 Oct. 56.3 54.8 Nov. 50.7 48.9 Dec. 48.7 47.4	.93 .93 .94 .93 .92 .91 .92	1 on 16 Aug. 3 " 21 " 1 " 5 Sept. 1 " 14 " 1 " 20 " 1 " 11 Nov. 1 " 2 Dec.	1 3 1 1 1 1 1	61 66 81 90 96 148 169	93 35	Cocoons opened, 5 dead fleas
"	14	Lab. Cupboard	June 66.4 58.2 July 71.5 62.7 Aug. 72.0 64.1 Sept. 65.7 55.7	.79 .75 .74 .77	1 " 21 July 1 " 27 " 1 " 2 Aug. 1 " 9 " 2 " 16 " 4 " 30 " 1 " 22 Sept.	1 1 1 1 2 4 1	35 41 47 54 71 75 98	21	3 cocoons opened 21 Oct. contained 1 larva died in pupation, 1 dried up flea, 1 dried up pupa
19	14	Warm Cupboard	69.8	.66	2 " 10 July 1 " 13 " 2 " 15 " 3 " 17 " 3 " 26 " 1 " 31 " 1 " 14 " 2 " 18 " 6 " 24 " 3 " 29 " 1 " 2 Aug. 2 " 5 July 2 " 10 " 2 " 11 " 1 " 13 " 1 " 8 Aug.	2 1 2 3 3 1 1 2 6 3 1 2 2 2 1 1	21 24 26 28 37 42 25 29 35 40 44 14 19 20 22 48	29 14	2 cocoons opened 24 Oct. contained 1 dried up flea, 1 dried up larva
"	13	Beehive	June 73.0 56.5 July 80.3 58.6 Aug. 80.2 57.6	—	1 " 14 " 2 " 18 " 6 " 24 " 3 " 29 " 1 " 2 Aug. 2 " 5 July 2 " 10 " 2 " 11 " 1 " 13 " 1 " 8 Aug.	1 1 2 3 1 2 2 2 1 1	25 29 35 40 44	35	Nil
21	16	Incubator 75 Dry	76	.57	2 " 5 July 2 " 10 " 2 " 11 " 1 " 13 " 1 " 8 Aug.	2 2 2 1 1	14 19 20 22 48	22	8 cocoons opened 23 Oct. contained 2 dried up pupae, 6 dried up larvae
26	14	" 75 Wet	76.1	.86	7 " 10 July 2 " 11 " 2 " 13 " 1 " 17 "	7 2 2 1	14 15 17 21	15	2 cocoons opened 2 Oct. and found to be empty ("false" cocoons)



[illegible]

TABLE XXXV.—Continued.

Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temperature	Humidity	Date of emergence of fleas	Duration of cocoon stage		Aver. days	Mortality per cent.	Remarks
						No. of cocoons	Days			
23 Sept. '11	22	Incubator 85 Wet	85.1	.77	4 on 7 Oct. 3 " 10 " 1 " 14 "	4 3 1	14 17 21	16	14	14 cocoons opened contained 2 dead fleas, 1 dead larva, 11 empty, "false" cocoons
"	22	" 85 Dry	85	.59	1 " 2 " 7 " 5 " 3 " 19 "	1 7 3	9 12 26	15	?Nil	Remaining cocoons empty, "false" cocoons, no cast skin
25 "	22	" 75 "	74.6	.52	4 " 14 " 4 " 19 " 2 " 23 " 2 " 31 "	4 4 2 2	19 24 28 36	25	45	4 larvae emerged from their cocoons and died at start; 6 cocoons opened 23 Oct. contained 5 dried up larvae, 1 dried up flea
"	22	" 75 Wet	75	.70	8 " 14 " 7 " 19 " 2 " 24 " 1 " 5 Dec. 1 " 28 "	8 7 2 1 1	19 24 29 71 94	28	Nil	3 "false" cocoons found
1 Dec. '11	22	" 93 "	92.3	.67	6 " 11 " 2 " 13 " 6 " 16 " 1 " 19 "	6 2 6 1	10 12 15 18	13	9	Cocoons opened 11 May '12, 2 dead fleas
4 "	22	" 93 Dry	92.8	.57	5 " 13 " 2 " 16 " 7 " 19 " 1 " 22 "	5 2 7 1	9 12 15 18	13	18	Cocoons opened 16 March '12, 4 dead fleas
"	22	" 75 Wet	75.8	.65	2 " 16 " 5 " 19 " 1 " 22 " 7 " 23 " 1 " 24 " 2 " 25 "	2 5 1 7 1 2	12 15 18 19 20 21	17	5	Cocoons opened 5 Sept. '12, 1 dead flea, 3 empty ("false" cocoons)
"	22	" 75 Dry	74.7	.51	5 " 16 " 3 " 19 " 1 " 22 " 2 " 25 "	5 3 1 2	12 15 18 21	15	27	One larva came out of cocoon and died; cocoons opened 11 May '12, 6 dead larvae
"	22	Warm Cupboard	Dec. 62.3 Jan. 59.7	.62 .60	1 " 22 " 3 " 24 " 4 " 25 " 3 " 4 Jan. 1 " 8 " 1 " 15 "	1 3 4 3 1 1	18 20 21 31 35 42	25	36	Cocoons opened 4 July '12, 8 dried up fleas, 1 "false" cocoon

7	"	22	Beehive	max. min. Dec. 46.8 35.0 Jan. 43.6 34.0 Feb. 46.9 34.3	—	None emerged	—	—	—	100	2 larvae came out of their cocoons and died; cocoons opened 4 July '12 contained 18 dried up larvae, 2 "false" cocoons
"	"	22	Cellar	Dec. 48.7 47.4 Jan. 47.2 45.9 Feb. 45.8 44.6	.92 .92 .92	None emerged	—	—	—	100	Cocoons opened 5 July '12 contained 19 dried up larvae, 3 "false" cocoons
"	"	22	Lab. Cupboard	Dec. 53.9 46.2 Jan. 52.5 44.9	.87 .89	None emerged	—	—	—	100	2 larvae came out of their cocoons and died; cocoons opened 3 July '12, 1 dried up pupa, 19 dried up larvae
6 April '12		22	Incubator 93 Dry	93.7	.63	2 on 15 April 1 " 20 " 2 " 22 "	2 1 2	9 14 16	13	27	Remaining cocoons opened 6 Sept., 2 dead fleas, 4 dead larvae, 15 empty (11 "false" cocoons)
9	"	23	" 93 Wet	93.1	.70	3 " 18 " 4 " 20 " 7 " 23 " 1 " 25 "	3 4 7 1	9 11 14 16	12	30	Remaining cocoons opened 4 Sept., 2 dead fleas, 1 dead pupa, 4 dead larvae (1 "false" cocoon)
12	"	22	" 75 "	April 75.8 May 75.6 June 74.9	.79 .86 .83	11 " 27 " 2 " 30 " 4 " 6 May 1 " 10 " 1 " 28 June	11 2 4 1 1	15 18 24 28 77	21	5	Remaining cocoons opened 5 Sept., 1 dead flea (2 "false" cocoons)
13	"	22	" 75 Dry	April 74.1 May 74.7	.56 .59	None emerged	—	—	—	100	4 larvae came out of cocoons and died; remaining cocoons opened 5 Sept., 17 dead larvae (1 "false" cocoon)
"	"	22	Cellar	max. min. April 50.3 49.1 May 51.2 53.2	.93 .93	None emerged	—	—	—	100	Cocoons opened 5 July '12, 19 dried up larvae, 3 "false" cocoons
15	"	22	Lab. Cupboard	April 60.6 50.4 May 64.0 56.8	.83 .87	1 on 25 May	1	40	—	95	Remaining cocoons opened 6 July '12, 16 dried up larvae, 4 dried up pupae, 1 dried up flea
"	"	22	Beehive	April 64.1 37.3 May 67.9 47.3	—	None emerged	—	—	—	100	Cocoons opened 4 July '12, 21 dried up larvae, 1 "false" cocoon
"	"	22	Warm Cupboard	April 59.0 May 62.6	.64 .70	2 on 20 May 1 " 31 "	2 1	35 46	—	77	Cocoons opened 4 July '12, 4 dried up pupae, 12 dried up larvae, 3 "false" cocoons
10 May '12		50	Incubator 75 Wet	May 75.6 June 74.9 July 75.0	.86 .83 .86	13 " 24 " 19 " 28 " 10 " 31 " 5 " 13 June 1 " 26 " 2 " 8 July	13 19 10 5 1 2	14 18 21 34 47 58	21	Nil	—
"	"	50	" 75 Dry	May 74.7 June 75.8	.59 .54	7 " 28 May 8 " 31 " 5 " 3 June 2 " 6 "	7 8 5 2	18 21 24 27	21	50	1 larva emerged from its cocoon and died; remaining cocoons opened 5 Sept. 23 dead larvae, 1 dead pupa (3 "false" cocoons)

TABLE XXXV.—Continued.

Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temperature	Humidity	Date of emergence of fleas	No. of cocoons	Duration of cocoon stage Days	Aver. days	Mortality per cent.	Remarks
10 May '12	50	Incubator 93 Wet	94.1	.76	5 on 20 May 14 " 24 " 8 " 28 " 1 " 31 "	5 14 8 1	5 14 8 1	14	42	Remaining cocoons opened 5 Sept., 15 dead fleas, 5 dead pupae, 1 dead larva (1 "false" cocoon)
13 "	50	" 93 Dry	93.3	.52	3 " 24 " 3 " 28 "	3 3	3 3	13	84	Remaining cocoons opened 5 Sept., 23 dried up larvae, 10 dried up pupae, 9 dried up fleas (2 "false" cocoons)
" "	50	Cellar	max. min. May 54.2 53.2 June 56.4 55.6 July 60.1 59.3 Aug. 57.5 56.5 Sept. 56.0 54.3	.93 .93 .93 .93 .92	1 " 5 July 1 " 26 " 1 " 13 Aug. 1 " 30 " *1 " 7 Sept.	1 1 1 1 1	1 1 1 1 1	74	86	Remaining cocoons opened 7 Sept. * 1 living flea, 18 dead larvae, 22 dead pupae, 3 dead fleas (2 "false" cocoons)
" "	50	Lab. Cupboard	May 64.0 56.8 June 64.6 58.3 July 68.5 62.1 Aug. 63.3 56.3 Sept. 61.5 53.0	.87 .81 .79 .84 .86	2 " 27 June 4 " 3 July 12 " 15 " 1 " 20 " 2 " 27 " 1 " 2 Aug. 4 " 9 " 1 " 14 " 1 " 28 " 1 " 30 " 2 " 6 Sept.	2 4 12 1 2 1 4 1 1 1 2	2 4 12 1 2 1 4 1 1 1 2	72	32	1 larva emerged from cocoon and died; remaining cocoons opened 6 Sept., 8 dried up larvae, 5 dried up pupae, 3 dried up fleas, 2 living fleas, 1 living pupa
" "	50	Beehive	May 67.9 47.3 June 67.6 49.9 July 73.4 55.4	—	1 " 20 June 4 " 27 " 3 " 4 July 1 " 20 " 1 " 26 " 1 " 3 June 9 " 8 " 6 " 12 " 7 " 21 " 1 " 27 " 4 " 2 July 1 " 10 " 2 " 12 " 1 " 20 " 1 " 28 " 5 " 3 June 13 " 6 " 9 " 8 " 6 " 12 "	1 4 3 1 1 1 9 6 7 1 4 1 2 1 1 5 13 9 6	1 4 3 1 1 1 9 6 7 1 4 1 2 1 1 5 13 9 6	51	72	3 larvae emerged from cocoons and died; remaining cocoons opened 6 Sept., 23 dead larvae, 8 dead pupae, 3 dead fleas (3 "false" cocoons)
16 "	50	Warm Cupboard	May 62.6 June 65.0 July 67.3	.70 .73 .71	1 " 3 June 9 " 8 " 6 " 12 " 7 " 21 " 1 " 27 " 4 " 2 July 1 " 10 " 2 " 12 " 1 " 20 " 1 " 28 " 5 " 3 June 13 " 6 " 9 " 8 " 6 " 12 "	1 9 6 7 1 4 1 2 1 1 5 13 9 6	1 9 6 7 1 4 1 2 1 1 5 13 9 6	36	26	4 larvae came out of cocoons and died; remaining cocoons opened 5 Sept., 2 dead fleas, 7 dead larvae (4 "false" cocoons)
24 "	50	Incubator 93 Wet	94.0	.78	5 " 3 June 13 " 6 " 9 " 8 " 6 " 12 "	5 13 9 6	5 13 9 6	14	32	Remaining cocoons opened 5 Sept., 16 dried up fleas (1 "false" cocoon)

"	"	50	"	93 Dry	May 93.3 June 93.0	.58 .55	None emerged	—	—	—	100	11 larvae emerged from cocoons and died; cocoons opened 5 Sept., 13 dried up fleas, 26 dried up pupae
"	"	50	"	75 Wet	May 75.6 June 74.9 July 75.0	.86 .83 .86	7 on 6 June 6 " 8 " 12 " 12 " 17 " 17 " 2 " 21 " 2 " 24 " 2 " 24 July	7 6 12 17 2 2 2	13 15 19 24 28 31 61	22	2	Cocoons opened 5 Sept., 1 dead flea (1 "false" cocoon)
28 May '12	"	50	"	75 Dry	75.8	.57	1 " 8 June 12 " 12 " 11 " 17 " 6 " 21 "	1 12 11 6	11 15 20 24	18	32	3 larvae emerged from cocoons and died; remaining cocoons opened 5 Sept., 2 dead fleas, 1 dead pupa, 10 dead larvae (4 "false" cocoons)
"	"	50	Cellar		max. min. June 56.4 55.6 July 60.1 59.3 Aug. 57.5 56.5 Sept. 56.0 54.3	.93 .93 .93 .92	3 " 15 July 1 " 19 " 1 " 26 " 2 " 3 Aug. 3 " 9 " 5 " 13 " 2 " 24 " 2 " 30 " *4 " 7 Sept.	3 1 1 2 3 5 2 2 4	48 52 59 67 73 77 88 94 102	76	26	Remaining cocoons opened 7 Sept., 10 dead pupae, 13 dead larvae * 4 living fleas (4 "false" cocoons)
"	"	50	Lab. Cupboard		June 64.6 58.3 July 68.5 62.1 Aug. 63.3 56.3 Sept. 61.5 53.0	.81 .79 .84 .86	4 " 3 July 2 " 15 " 3 " 20 " 8 " 27 " 2 " 2 Aug. 5 " 9 " 1 " 14 " +3 " 6 Sept.	4 2 3 8 2 5 1 3	36 48 53 60 66 73 78 101	62	(?) 18	Cocoons opened 6 Sept., 8 dead larvae, 1 dead pupa + 3 living fleas, remainder of cocoons lost through an accident
"	"	50	Beehive		June 67.7 49.9 July 73.4 55.4 Aug. 64.9 45.6	—	1 " 27 June 1 " 4 July 4 " 15 " 14 " 20 " 5 " 26 " 1 " 2 Aug. 1 " 11 " 1 " 24 "	1 1 4 14 5 1 1 1	30 37 48 53 59 66 75 88	54	38	Remaining cocoons opened 6 Sept., 12 dead larvae, 4 dead fleas, 3 dead pupae (3 "false" cocoons)
"	"	50	Warm Cupboard		June 65.0 July 67.3	.73 .71	17 " 21 June 12 " 27 " 4 " 2 July 1 " 12 " 2 " 20 " 1 " 26 "	17 12 4 1 2 1	24 30 35 45 55 61	33	20	2 larvae emerged from their cocoons and died; cocoons opened 5 Sept., 6 dead larvae, 2 dead fleas (3 "false" cocoons)

TABLE XXXV.—Continued.

Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temperature	Humidity	Dates of emergence of fleas	No. of cocoons	Duration of cocoon stage Days	Aver. days	Mortality per cent.	Remarks
3 June '12	50	Incubator 75 Wet	75.1	.83	1 on 12 June 23 " 17 " 9 " 21 " 13 " 24 " 1 " 28 "	1 23 9 13 1	1 23 9 21 25	17	Nil	Remaining cocoons opened 5 Sept. empty ("false" cocoons)
" "	25	"	75.8	.59	16 " 17 " 10 " 21 " 2 " 25 "	16 9 1	14 18 17	16	44	6 larvae emerged from their cocoons and died; cocoons opened 5 Sept., 13 dead larvae, 1 dead pupa, 2 dead fleas
3 "	50	"	94.2	.78	3 " 12 " 3 " 17 " 4 " 21 " 1 " 24 "	3 3 4 1	9 14 18 21	14	52	Remaining cocoons opened 4 Sept., 2 dead fleas, 15 dead pupae, 9 dead larvae, 13 "false" cocoons (?)
" "	50	"	93.0	.54	4 " 12 " 2 " 17 "	4 2	9 14	11	82	7 larvae emerged from their cocoons and died: remaining cocoons opened 5 Sept., 4 dead fleas, 19 dead pupae, 11 dead larvae, 3 "false" cocoons (?)
6 "	42	Cellar	max. min. June 56.4 55.6 July 60.1 59.3 Aug. 57.5 56.5 Sept. 56.0 54.3	.93 .93 .93 .92	4 " 26 July 6 " 9 Aug. 3 " 13 " *2 " 7 Sept. 1 " 14 Oct. *2 " 14 "	4 6 3 2 3	50 64 68 93 130	76	Doubtful	23 cocoons opened 7 Sept., 17 empty, 2 dead fleas, 2 dead pupae, 2 live fleas*; 19 cocoons opened 14 Oct., 13 empty, 2 dead fleas, 2 dead pupae, 1 dead larva, 2 live fleas* ("false" cocoons unrecorded, presumably 15)
12 July '12	50	Incubator 75 Wet	July 75.0 Aug. 74.3	.86 .81	20 " 24 July 20 " 28 " 2 " 5 Aug.	20 20 2	12 16 23	14	14	Cocoons opened 5 Sept., 5 dead larvae, 2 dead pupae (1 "false" cocoon)
" "	50	"	July 76.2 Aug. 75.0	.64 .56	8 " 24 July 8 " 25 " 14 " 28 " 7 " 31 " 1 " 3 Aug.	8 8 14 7 1	12 13 16 19 22	15	14	Remaining cocoons opened 5 Sept., 3 dead fleas, 3 dead pupae, 1 dead larva (5 "false" cocoons)
" "	50	"	95.3	.79	5 " 24 July	—	—	12	76	Cocoons opened 4 Sept., 13 dried up fleas, 16 dried up pupae, 9 dried up larvae (? 7 "false" cocoons)
" "	50	"	93.0	.58	16 " 20 " 1 " 24 " 1 " 28 " 1 " 31 "	16 1 1 1	8 12 16 19	9	60	Cocoons opened 5 Sept., 4 dead fleas, 12 dead pupae, 14 dead larvae (1 "false" cocoon)

TABLE XXXVI. Cocoons *P. irritans*. "Continuous"\* series, reared from eggs, each batch maintained during egg-larval and cocoon stage in the same situation.

Incubator 75° Wet.									
Date of commencement of experiment in the egg (or larval) stage	No. of cocoons found	Temp.	Humidity	Approximate duration of cocoon stage	Average no. of days in cocoon	Mortality in cocoon stage	Food	Remarks	
13 July '10	2	75.0	Unrecorded	1 8 1 13	10	Nil	Rat faeces	—	
19 "	3	75.0	.67	1 8 1 11	9	33 0/10	Dead flies	—	
11 Aug. '10	4	75.0	.60	4 12	—	Nil	B.S. Rag	—	
1 Oct. '10	1	74.4	.73	1 15	—	Nil	B.S. Rag	—	
27 Mar. '11	13	75.1	.80	1 9 1 10 1 12 3 13 1 14 2 15 1 16	13	Nil	Rat faeces crushed	3 opened and found to be empty ("false" cocoons)	
9 June '11	20	76.0	.84	1 9 1 11 3 12 4 13 4 15 4 17 1 18 1 19 1 34	15	Nil	B.S. Rag	—	
9 "	6	76.1	.87	3 11 1 16 1 22	14	Nil	Flea faeces	One cocoon opened empty ("false" cocoon)	

\* Experiments in Table XXXVI are for the most part in direct continuity with those dealing with the larval stage given in Table XXI, and up to 15 February 1911 with egg stage experiments given in Table V.

TABLE XXXVI.—Continued.

Date of commencement of experiment in the egg (or larval) stage		Incubator 85° Wet.									
		Temp.	Humidity	No. of cocoons	Approximate duration of cocoon stage Days	Average no. of days in cocoon	Mortality in cocoon stage	Food	Remarks		
24 July '10	1	about 85.0	.72	1	23	—	Nil	B.S. Rag	—		
6 Aug. '10	1	84.3	.75	1	9	—	Nil	B.S. Rag	—		
26 Sept. '10	7	84.4	.72	1	6	7	43 0/10	Flea faeces	—		
				2	7						
				1	11						
29 March '11	15	84.0	.74	1	6	12	11 0/10	Rat faeces crushed	Remainder (7 cocoons) opened, one found to contain dried up flea, remainder "false" cocoons		
				1	8						
				3	12						
				2	14						
				1	21						
6 June '11	18	84.0	.81	1	7	13	Nil	B.S. Rag	2 cocoons opened and found empty ("false" cocoons)		
				1	8						
				3	9						
				2	10						
				3	11						
				2	14						
				2	16						
				2	26						
7 "	9	84.2	.80	1	9	13	Nil	Flea faeces	1 cocoon opened and found empty ("false" cocoon)		
				2	11						
				4	12						
				1	26						



		Laboratory Cupboard.									
13 July '10	1	60	.84	1	22	—	Nil	Rat faeces	—		
1 Aug. '10	1	60	.84	1	43	—	Nil	Flea faeces	—		
11 "	6	Approx. only to 8 Nov. 60	.84	1	23	116	Nil	B.S. Rag	—		
		max. min.		1	24						
		Nov. 54.0 42.8	.84	1	104						
		Dec. 56.5 47.8	.85	1	114						
		Jan. 53.5 44.0	.84	1	215						
		Feb. 52.8 43.6	.83	1	220						
		Mar. 55.2 45.5	.85								
		Apr. 58.2 48.2	.78								
14 "	3	Approx. 60	.84	1	59	—	Nil	—	—		
20 "	10	Approx. to 8 Nov. 60	.84	1	37	75	Nil	Flea faeces	22 July '11 5 opened and found to be empty (? false cocoons)		
		max. min.		1	40						
		Nov. 54.0 42.8	.84	1	55						
		Dec. 56.5 47.8	.85	1	66						
		Jan. 53.5 44.0	.84	1	178						
		Feb. 52.8 43.6	.83	5 opened							
		Mar. 55.2 45.5	.85								
		Apr. 58.2 48.2	.78								
1 Oct. '10	16	(as above)		3	74	83	31 0/0	B.S. Rag	24 Aug. '11 5 opened and found to contain dried up larvae		
				1	77						
				4	81						
				1	86						
				1	87						
				1	113						
				5 opened							
2 "	2	—	—	1	143	—	50 0/0	B.S. Rag	1 cocoon opened 21 Oct. '11 found to contain dried up pupa		

TABLE XXXVI.—Continued.

Date of commence- ment of experiment in the egg (or larval) stage	No. of cocoons found	Temp. (as above to April)	Humidity	Approximate duration of cocoons stage		Average no. of days in cocoon	Mortality in cocoon stage	Food	Remarks
				1	172				
10 "	2	May 61.8 54.3 June 66.4 58.2	.78 .79			—	—	Bran	—
3 June '11	7	72.4 61.1	.73	3	13	17	Nil	B.S. Rag	—
				2	19				
				2	20				
12 "	13	71.1 63.1	.74	5	13	17	Nil	Flea faeces	—
				1	14				
				2	18				
				1	20				
				4	21				
14 Oct. '10	2	1911 max. min. Feb. 46.4 44.8 Mar. 46.5 45.3 Apr. 48.5 46.6 May 53.9 52.6	.92 .91 .91 .92	1	38	Cellar.	Nil	Bran	—
				1	90	64			
June '11	18	June 58.1 56.7 July 61.6 60.4 Aug. 64.5 63.4 Sept. 60.3 59.5	.93 .93 .94 .93	1	13	19	11%	B.S. Rag	2 opened 8 Nov. '11 contained 1 dried up larva, 1 dried up flea
				3	18				
				3	19				
				8	20				
				1	26				
				2 opened					
12 "	17	—	—	4	6*	27	6%	Flea faeces	1 cocoon opened 9 Nov. '11 con- tained living flea; 1 cocoon, opened 14 Dec. 1911, contained a dead flea
				1	13				
				2	18				
				2	19				
				1	20				
				4	26				
				1	68				
				1	111				

\* Period may be 3 or 4 days longer owing to lapsus in observation.

TABLE XXXVII. Cocoons *P. irritans*. "Continuous" series, contrasted experiments (compiled from Table XXXVI); larvae from same batch of eggs.

Date of commencement of experiment in egg stage	Place	No. of cocoons found	Temp.	Humidity	Approximate duration of cocoon stage No. of cocoons Days	Av. no. of days in cocoon	Mortality in cocoon stage	Food
11 Aug. '10	Incubator 75 Wet	4	75.0	.60	4 12	12	Nil	B.S. Rag
11 "	Laboratory Cupboard	6	Approximate only to 8 Nov. 60 max. min. Nov. 54.0 42.8 Dec. 56.5 47.8 Jan. 53.5 44.0 Feb. 52.8 43.6 Mar. 55.2 45.5 Apr. 58.2 48.2	.84	1 23 1 24 1 104 1 114 1 215 1 220	116	Nil	B.S. Rag

TABLE XXXVIII. Cocoons *P. irritans*. Larvae all reared and cocoons spun in Incubator 75 Wet; cocoons then removed to various situations.

Date on which cocoons were placed under test conditions	No.	Place	Temp.		Humidity	Dates of emergence of fleas	Approximate duration of cocoon stage	Mortality	Remarks
			max.	min.			No. of cocoons		
(a) Jan.—April 1911. Temperature of Incubator 75 Wet, 74.4, Humidity 77 for Jan., 70 for March and April.									
3 Jan. '11	16	Beehive	Jan. 43.9	32.0	—	1 on 21 March	1	77	93%
			Feb. 43.8	30.7	—				
			Mar. 52.5	33.3	—				
2 March	2	Incubator 85 Wet	83.9		.71	3 11 "	2	7	8
4 "	3					1 12 "	1	8	
							1	9	
12 "	4	" 85 Dry	84.0		.58	1 22 "	1	10	12
						1 27 "	1	15	50
									2 cocoons opened 19 Sept., 1 contained dried up larva
20 "	2	Lab. Cupboard	Mar. 52.2	45.5	.85	1 Nil	2	43	58
19 April	5		Apr. 58.2	48.2	.78	1 27 "	1	45	
			May 61.8	54.3	.78	2 2 June	1	52	
			June 66.4	58.2	.79	1 10 "	1	68	
			July 71.5	62.7	.75	1 27 July	1	99	
27 March	3	Cellar	Mar. 46.5	45.3	.91	1 26 June	1	91	116
			Apr. 48.5	46.6	.91	1 16 Aug.	1	142	33
			May 53.9	52.6	.92				Remaining cocoon opened contained dead flea
			June 58.1	56.7	.93				
			July 61.6	60.4	.93				
			Aug. 64.5	63.4	.94				
15 April	8	Incubator 75 Dry	75.2		.50	1 23 April	1	8	14
						1 27 "	1	12	
						1 28 "	1	13	
						1 30 "	1	15	
						1 9 May	1	24	
19 "	5	Warm Cupboard	Apr. 63.0		.57	1 29 "	1	40	44
			May 62.9		.60	1 30 "	1	41	
			June 66.6		.65	1 2 June	1	43	
						1 7 "	1	48	
						1 9 "	1	50	
									38
									Remaining cocoons opened contained dried up larvae

(b) Special experiment with cocoons taken from the same batch; larvae reared and cocoons spun in Incubator 75° Wet. (Temp. April—May 1911, 75° F., Humidity 82).

10 May '11	10	Incubator 75° Dry	75.0	.53	1 on 21 May	11	14	Nil	5 cocoons opened 16 May contained 4 living pupae and 1 larval skin
					1 22 "	12			
					2 24 "	14			
					1 26 "	16			
					1 29 "	19			
11—12 May	12	" 85° Dry	83.9	.60	3 21 "	9	10	8	1 cocoon opened 23 Oct. contained dried up larva
					5 22 "	10			
					1 24 "	12			
					2 26 "	14			
13—15 "	11	" 85° Wet	84.0	.76	5 22 "	7	9	Nil	1 cocoon opened 2 Oct. empty ("false cocoon")
					3 25 "	10			
					2 26 "	11			
20—22 "	18	" 75° Wet	76.0	.86	6 1 June	10	18	Nil	Remaining cocoons opened 2 Oct. empty ("false" cocoons)
					2 2 "	11			
					4 6 "	15			
					1 30 "	39			
					1 2 Aug.	72			
16—19 "	17	Lab. Cupboard	May 65.0 56.5 June 66.4 58.2	.78 .79	8 10 June	22	25	20	5 cocoons opened 21 Oct., 1 contained dried up larva, 2 contained dried up pupae, 2 empty "false" cocoons
					2 18 "	30			
					1 20 "	32			
					1 27 "	39			
16—19 "	16	Cellar	May 53.9 52.6 June 58.1 56.7 July 61.4 60.4 Aug. 64.5 63.4 Sept. 60.6 59.5	.92 .93 .93 .94 .93	1 5 July	47	139	Nil	Remaining cocoons opened 21 June '12, 2 "false" cocoons
					1 27 "	69			
					1 9 Aug.	82			
					2 16 "	89			
					1 21 "	94			
					1 5 Sept.	109			
					1 16 Oct.	150			
					1 20 Nov.	170			
					1 22 Dec.	202			
					3 25 "	205			
					1 25 Jan.	236			
20—22 "	12	Beehive	May 76.0 48.0 June 73.0 56.5 July 74.1 55.4	— — —	2 6 June	15	26	16	2 cocoons opened 25 Oct. contained 1 dried up flea, 1 dried up larva
					5 10 "	19			
					1 20 "	29			
					1 14 July	53			
					1 18 "	57			





TABLE XXXVIII.—Continued.

(d) Special experiment with cocoons taken from the same batch; larvae reared and cocoons spun in Incubator 75 Wet. (Temp. August 1911, 75-7° F., Humidity .83).

Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temp. max. min.	Humidity	Dates of emergence of fleas	No. of cocoons	Approximate duration of cocoon stage Days	Aver.	Mortality	Remarks
1 Aug. '11	36	Cellar	Aug. 64.5 63.4 Sept. 60.3 59.5 Oct. 56.3 54.8 Nov. 50.7 48.9 Dec. 48.7 47.4 Jan. 47.2 45.9 Feb. 45.8 44.6 Mar. 49.1 48.1 Apr. 50.3 49.1	.94 .93 .92 .91 .92 .92 .93 .93	4 on 21 Aug. 3 28 " 2 11 Sept. 1 16 Oct. 5 1 Nov. 1 9 " 1 20 " 1 7 Dec. 4 20 " 1 22 " 1 4 Jan. 2 25 " 1 31 " 1 6 Feb. 2 13 " 1 26 " 1 27 March	4 3 2 1 5 1 1 1 4 1 1 2 1 2 1 1	20 27 41 76 92 100 111 128 141 143 156 177 183 187 196 209 239	110	Nil	4 cocoons missing, probably they were slightly spun and deserted by the larvae and subsequently fell to pieces
5 "	39	Lab. Cupboard	Aug. 72.0 64.1 Sept. 65.7 55.7 Oct. 59.0 50.9 Nov. 54.9 46.1 Dec. 53.9 46.2 Jan. 52.5 44.9 Feb. 54.4 45.3	.74 .77 .84 .85 .87 .89 .86	8 16 Aug. 18 30 " 1 9 Sept. 1 13 Oct. 1 20 " 1 22 " 1 4 Jan. 1 17 Feb.	8 18 1 1 1 1 1 1	11 25 34 69 76 124 139 152 196	40	10	Remaining cocoons opened 3 July '12 contained 1 dried up flea, 3 dried up larvae, 2 false cocoons
8 "	33	Beehive	Aug. 80.2 57.6 Sept. 75.3 48.0	— —	7 17 Aug. 12 22 " 1 26 " 1 28 " 1 9 Sept. 1 16 " 1 22 "	7 12 1 1 1 1 1	9 14 18 20 32 39 45	16	3	Remainder of cocoons opened 23 June '12 contained 1 dead larva, 8 false cocoons



11	"	18	Warm Cupboard	Aug. 75.0 Sept. 66.3 Oct. 67.4	.66 .66 .59	8 4 1 1 1	22 Aug. 25 " 8 Sept. 30 " 23 Oct.	8 4 1 1 1	11 14 28 50 73	19	17	Remainder of cocoons opened 13 Dec. contained 3 dried up larvae
14	"	26	Incubator 75 Dry	74.9	.59	14 2 5	25 Aug. 28 " 4 Sept.	14 2 5	11 14 21	14	19	Remaining cocoons opened 13 Dec. contained 5 dried up larvae
18	"	53	" 85 Dry	85.7	.59	6 13 19 2 1	25 Aug. 28 " 4 Sept. 8 " 25 "	6 13 19 2 1	7 10 17 21 38	14	23	Remaining cocoons opened 16 Mar. '12 contained 2 dead fleas, 10 dead larvae
23	"	42*	" 85 Dry	84.2	.59	3 15 8 3 1 1	28 Aug. 4 Sept. 8 " 9 " 14 " 19 "	3 15 8 3 1 1	5 12 16 17 22 27	14	26	Remaining cocoons opened 16 Mar. '12 contained 2 dead fleas, 9 dead larvae
23	"	42*	Cellar	Aug. 64.5 63.4 Sept. 60.3 59.5 Oct. 56.3 54.8 Nov. 50.7 48.9 Dec. 48.7 47.4 Jan. 47.2 45.9 Feb. 45.8 44.6 Mar. 49.1 48.1 Apr. 50.3 49.1	.94 .93 .92 .91 .92 .92 .92 .93 .93	4 3 2 3 3 2 2 1 1 1 5 1 1 1 2 1 3 2 1	11 " 19 " 20 " 26 " 2 Oct. 16 " 1 Nov. 9 " 20 " 27 " 11 Dec. 20 " 22 " 25 " 10 Jan. 31 " 19 Feb. 2 March 10 April	4 3 2 3 3 2 2 1 1 1 5 1 1 1 2 1 3 2 1	19 27 28 34 40 54 70 78 89 96 110 119 121 124 140 161 180 192 231	89	2	Remaining cocoons opened 21 June '12 contained 1 dead flea, 1 "false" cocoon

\* Refer to the same batch of cocoons; the batch was divided, half being placed in Incubator 85 Dry, the remainder being put into cellar for comparison.

TABLE XXXVIII.—Continued.

(e) Special experiment with cocoons taken from the same batch; larvae reared and cocoons spun in Incubator 75 Wet. (Temp. Nov.—Dec. 1911, 75° F., Humidity .70.)										
Date on which the cocoons were placed under test conditions	No. of cocoons	Place	Temp.	Humidity	Dates of emergence of fleas	No. of cocoons	Approximate duration of cocoon stage	Aver.	Mortality	Remarks
6 Nov. '11							Days			
16 "	16	Incubator 93 Dry	92.9	.56	2 on 13 Nov. 2 16 " 1 1 Dec.	2 2 1	7 10 25	12	69	Cocoons opened 16 March '12 contained 7 dead fleas, remainder 4 dead larvae
16 "	22	" 85/93 Wet	84.2 93.0	.72* .66	2 29 Nov.	2	13	—	Doubtful	Cocoons opened 11 May '12 contained 6 dead pupae, 4 dead larvae, 10 unaccounted for, presumably false cocoons
17 "	16	" 75 Dry	74.5	.56	2 5 Dec.	2	18	—	87	Cocoons opened 13 Dec. contained 14 dried up larvae
21 "	19	" 75 Wet	Nov. 75.0 Dec. 75.8 Jan. 75.5 Feb. 75.0 Mar. 75.1 Apr. 75.8 May 75.6	.69 .65 .71 .76 .79 .79 .86	2 1 " 7 5 " 1 16 " 1 24 " 1 1 Jan. 1 16 May	2 2 1 1 1 1	10 14 25 33 41 146	28	21	Cocoons opened 4 July '12 contained 2 dried up fleas, 2 dried up larvae, 2 false cocoons
21 "	19	Warm Cupboard	Nov. 63.0 Dec. 62.3 Jan. 59.7 Feb. 59.5	.58 .62 .60 .63	2 5 Dec. 1 7 " 2 11 " 2 13 " 1 18 " 1 22 " 1 26 " 1 4 Jan. 2 8 " 1 25 " 1 12 Feb.	2 1 2 2 1 1 1 1 2 1 1	14 16 20 22 27 31 35 44 48 65 83	34	10	Cocoons opened 4 July '12 contained 2 dried up larvae, 1 false cocoon
23 "	17	Beehive	Nov. 23 to 30 } 42.0 32.7 Dec. 46.8 35.0	— —	No emergence			—	100	Cocoons opened 23 June '12; many of the cocoons very frail and in fragments, apparently there had been 5 false cocoons. There were 12 dead larvae

Thermometer fell to 23° for three nights, Dec. 6—8



TABLE XXXVIII.—Continued.

(g) Emergence of adults from stock of cocoons kept in Laboratory Cupboard, larvae reared and cocoons spun in Laboratory Cupboard.

Dates when cocoons were found	Temperature max. min.	Humidity	Dates of emergence	Average 143 days	Approx. no. of days in cocoon
1 on 15 Sept. '10	Sept. '10 about 60	.84	1 on 27 Jan. '11		1 in 134
3 21 "	Oct. "	.84	4 27 Feb. '11		* { 1 155
2 25 "	Nov. 54.0 42.8	.84	2 1 March '11		1 157
Remainder during October	Dec. 56.5 47.8	.85	1 2 "		3 { 1 159
	Jan. '11 55.5 44.0	.84	2 3 "		1 121
	Feb. 52.8 43.6	.83	2 4 "		1 122
	March 55.2 45.5	.85	1 9 "		1 123
	April 58.2 48.2	.78	2 20 "		2 124
			1 23 "		1 129
			1 1 April '11		+ { 2 140
			1 18 "		1 143
			1 21 "		1 151
					1 169
					1 172

\* These were assumed to have emerged from the Sept. cocoons.

+ All dates calculated from 31 Oct. '11. One cocoon was kept apart so that its date of emergence can be definitely fixed at 169 days.

TABLE XXXIX. Cocoons *P. irritans*. Larvae reared in Incubator 75° Wet (Temperature 75° F., Humidity 78) these allowed to spin under one set of conditions and transferred to contrasting conditions for the cocoon stage.

Date on which larvae were re-moved from In-cubator	No. of larvae	Place in which larvae formed their cocoons and date when found and transferred	Temperature max. min.	Humidity	Place to which cocoons were transferred and date of emergence of fleas	Temperature max. min.	Humidity	No. of cocoons	Approximate duration of cocoon stage Days	Aver. Mortality	Remarks
27 April '11	10	Beehive 1 18 May 1 26 " Remainder of larvae dead	69.1 43.7	—	Incubator 85° Wet 1 29 May 1 6 June	84.7	80	2	11	— Nil	—
27 April	10	Incubator 85° Wet 2 2 May 3 4 " 3 5 " Remainder of larvae dead	83.5	.75	Beehive 2 30 May 2 16 June 1 20 "	max. min. May 71.8 45.2 June 73.0 56.5	—	2 2 1	28 43 47	37 0/0	3 cocoons opened 30 Sept. contained 1 dried up flea, 2 dried up larvae
2 May	10	Lab. Cupboard 1 9 May 2 13 " 1 23 " 1 27 " 1 1 June 1 larva emerged from cocoon and failed to spin again Remainder of larvae dead	61.8 54.3	.78	Incubator 75° Dry 2 23 May 1 7 June 1 13 "	75.3	.53	1 1 1 1	10 14 21 25	20 0/0	Remaining cocoons opened 23 Oct. contained dried up larvae
27 April	10	Cellar 1 9 May 2 18 " 2 27 " 1 3 June 1 8 " 2 larvae emerged from cocoons and failed to spin again Remainder of larvae dead	54.8 53.4	.92	Incubator 85° Dry 1 20 June 1 2 Aug.	84.1	.61	1 1	41 76	60 0/0	Remaining cocoons opened 2 Oct. contained dried up larvae
2 May	10	Incubator 85° Dry 7 8 May 1 11 " Remainder dead	84.3	.59	Cellar 2 3 June 2 20 " 1 12 July 1 1 Aug.	May 53.9 52.6 June 58.1 56.7 July 61.6 60.4	.92 .93 .93	2 2 1 1	26 43 65 85	Nil	Remaining cocoons opened and found empty (false cocoons)
2 May	16	Incubator 75° Wet 4 5 May 9 8 " 3 10 " 1 12 "	74.4	.83	Warm Cupboard 1 19 May 1 21 " 1 23 " 1 24 " 1 26 " 2 29 " 4 31 " 1 1 June 2 6 " 1 8 "	May 62.9 June 66.6	.60 .65	1 1 2 1 2 4 1 1 2 2	14 16 18 19 21 23 24 27 29	12 0/0	1 larva came out of cocoon and died, 1 cocoon opened 30 Sept. contained dried up larva

TABLE XL. *Cocoon stage* *Ct. canis*.

(a) Larvae taken from dog's bed placed in large glass tubes and allowed to spin and to pass the cocoon stage in the same place (1) in Incubator 75 Wet and (2) in the Cellar.

Date on which larvae were put into tubes	No. of cocoons	Place to which cocoons were transferred	Temperature		Humidity	Date of emergence of fleas		Mortality	Remarks
26 April '11	17 cocoons found	Incubator 75 Wet	April	75.0	.78	1 on 8 May '11		*	—
			May	74.9	.85	3 10 "			
			June	75.8	.86	1 23 "			
						1 27 "			
						3 1 June '11			
						14 6 "			
						1 8 "			
"	28 cocoons found	Cellar	April	48.5	46.6	2 5 July '11		—	—
			May	53.9	52.6	7 12 "			
			June	58.1	56.7	4 3 Aug. '11			
			July	61.6	60.4	1 9 "			
			Aug.	64.5	63.4				

(b) Larvae taken from dog's bed and allowed to spin in Laboratory Cupboard, November 1910; cocoons then removed to various situations.

Date on which cocoons were placed under test conditions	No. of cocoons	Place	Temperature		Humidity	Date of emergence of fleas		Mortality	Remarks
17 Nov. '10†	7	Incubator 85 Wet		83.9	.71	1 23 Nov. '10		Nil	1 cocoon opened and found to be empty ("false" cocoon)
"	7	"		74.2	.73	5 25 "		14 0/0	2 cocoons opened found to be empty (1 dead flea, 1 "false" cocoon)
"	6	Warm Cupboard	max. min.	67.5 61.0	.54	2 4 Dec. '10		16 0/0	1 cocoon opened found to contain dead flea
"	7	Cellar		48.6 47.1	.92	1 13 Feb. '10		71 0/0	5 cocoons opened contained dead fleas
						1 18 "			

\* NOTE. No accurate figures of mortality can be given.

† There is some doubt as to the correct date of spinning, the date given is the date of transfer only, the number of days in cocoon is not definite.

(c) Larvae taken from dog's bed and allowed to spin in Incubator 75 Wet (Temperature 76° F., Humidity 84); cocoons then removed to various situations.

Date on which cocoons were placed under test conditions 31 July '11	No. of cocoons about 100	Place to which cocoons were transferred Incubator 75 Wet	Temperature	Humidity	Date of emergence of fleas	No. of cocoons	Approximate duration of cocoon stage Days	Average	Remarks
"	100	"	75 Dry	.59	85 on 15 Aug. '11 7 17 " " 18 " " 2 12 Oct. '11	85 7 1 2	15 17 18 72	16	Remaining cocoons opened 16 Dec. '11 contained dried up larvae
	100	"	75 Dry	.59	78 14 Aug. '11 1 25 " " 1 28 " " 1 4 Sept. '11	78 1 1 1	14 25 28 35	15	Remaining cocoons opened 13 Dec. '11 contained dried up fleas
	100	"	85 Wet	.79	77 14 Aug. '11 1 21 " " 1 28 " " 1 12 Sept. '11 1 19 " "	77 1 1 1 1	14 21 28 43 50	15	Remaining cocoons opened 16 Dec. '11 contained dried up fleas
	100	"	85 Dry	.61	80 14 Aug. '11 3 25 " " 1 19 Sept. '11	80 3 1	14 25 50	15	Remaining cocoons opened 16 Dec. '11 contained dried up fleas
"	100	Cellar						130	Remaining cocoons opened 7 Sept. '12 contained 2 dead fleas
			max. min.						
		Aug.	64.5	63.4	12 16 Aug. '11	12	16		
		Sept.	60.6	59.5	21 28 " " 9 11 Sept. '11	21	28		
		Oct.	56.3	54.8	9 19 " " 3 19 " " 1 16 Oct. '11	9	42		
		Nov.	50.7	48.9	3 19 " " 1 16 Oct. '11	3	50		
		Dec.	48.7	47.4	1 16 Oct. '11	1	80		
		1912			2 9 Nov. '11	2	101		
		Jan.	47.2	45.9	6 20 Dec. '11	6	142		
		Feb.	45.8	44.6	3 22 " " 2 25 " " 1 29 " " 4 4 Jan. '12	3	144		
		Mar.	49.1	48.1	2 25 " " 1 29 " " 4 4 Jan. '12	2	147		
		April	50.3	49.1	1 29 " " 4 4 Jan. '12	1	151		
		May	54.2	53.2	11 10 " " 6 Feb. '12	4	157		
		June	56.4	55.6	8 6 Feb. '12	11	163		
		July	60.1	59.3	1 19 " " 3 26 " " 6 21 Mar. '12	8	190		
					3 26 " " 5 27 " " 4 April '12	1	203		
					6 21 Mar. '12	3	210		
					5 27 " " 3 4 April '12	6	234		
					3 4 April '12	5	240		
					2 10 " " 4 26 " " 2 9 May '12	3	248		
					4 26 " " 2 25 " " 1 19 July '12	2	258		
					2 25 " " 2 25 " " 1 19 July '12	4	270		
					2 25 " " 2 25 " " 1 19 July '12	2	283		
					1 19 July '12	2	299		
						1	354		

TABLE XL.—Continued.

Date on which cocoons were placed under test conditions	No. of cocoons	Place to which cocoons were transferred	Temperature max. min.	Humidity	Date of emergence of fleas	No. of cocoons	Approximate duration of cocoon stage Days	Average	Remarks
31 July '11	about 100	Lab. Cupboard	Aug. 72.0 64.1 Sept. 65.7 55.7 Oct. 59.0 50.9 Nov. 54.9 46.1 Dec. 53.9 46.2 1912 Jan. 52.5 44.9 Feb. 54.4 45.3 Mar. 56.0 47.8 April 60.6 50.4	.74 .77 .84 .85 .87  .89 .86 .76 .83	80 on 16 Aug. '11 5 30 " 2 6 Sept. '11 1 19 Dec. '11 1 22 " 1 25 " 2 10 Feb. '12 1 17 " 3 20 " 1 23 " 3 5 Mar. '12 3 11 " 1 21 " 1 25 " 1 28 " 2 1 April '12 3 14 " 3 24 " 1 30 "	80 5 2 1 1 1 2 1 3 1 3 3 1 1 2 3 3 1	16 30 37 141 144 147 194 201 204 207 218 224 234 238 241 245 258 268 274	67	Cocoons opened, no dead found
"	100	Beehive	80.2 57.6	—	20 14 Aug. '11 48 18 " 26 22 " 1 26 "	20 48 26 1	14 18 22 26	18	Cocoons opened contained 7 dried up fleas
"	100	Warm Cupboard	Dry 75.0 66.3 67.4	.66 .66 .59	80 14 " 5 17 " 7 22 " 1 23 Sept. '11 1 25 " 1 12 Oct. '11	80 5 7 1 1 1	14 17 22 54 56 73	16	Remaining cocoons opened 13 Dec. '11 contained dried up fleas

NOTE. No accurate figures of mortality can be given.



(d) Larvae taken from a dog's bed and allowed to spin in Incubator 75 Wet; cocoons then removed to various situations.

Date on which cocoons were placed under test conditions	No. of cocoons	Place to which cocoons were transferred	Temperature	Humidity	Date of emergence of fleas	Number of days in cocoon	Average	Remarks
8 July '12	50 approx.	Incubator 93 Wet	94.8	.82	1 on 13 July '12 3 19 "	5 11	9	Cocoons opened 4 Sept. contained 4 dead fleas, 22 dead pupae, 11 dead larvae
12 "	50 "	" 93 Dry	93.0	.57	21 19 " 6 24 " 2 28 "	7 12 16	8	Cocoons opened 4 Sept. contained 10 dead fleas, 2 dead pupae, 6 dead larvae
12 "	50 "	" 75 Wet	75.0	.86	4 18 " 38 24 " 2 28 " 1 4 Aug. '12	6 12 16 23	12	Cocoons opened 5 Sept. all empty; probably no mortality
12 "	50 "	" 75 Dry	76.2	.64	2 19 July '12 39 24 " 2 28 " 1 1 Aug. '12	7 12 16 20	12	Cocoons opened 5 Sept. contained 1 dead larva, remainder empty
12 "	50 "	* Cellar	max. min. July 60.1 59.3 Aug. 57.5 56.5 Sept. 56.0 54.3	.93 .93 .92	+10 7 Sept. '12 1 28 " +2 11 Oct. '12 3 14 " +12 14 " 1 17 " 1 31 " 1 13 Nov. '12 1 21 " 1 23 " 1 19 Dec. '12 1 21 " 1 17 Feb. '13 1 25 Mar. '13 7 emerged between May and July	57 78 91 94 94 97 111 124 132 134 160 162 220 256 say 292	129	1 dead flea found in cocoon, remaining cocoons empty

\* No record of temperature or humidity kept subsequently to Sept. 1912.

† Taken out of cocoons.

NOTE. No accurate figures of mortality can be given.

TABLE XL.—Continued.

Date on which cocoons were placed under test conditions	No. of cocoons	Place to which cocoons were transferred	Temperature max. min.	Humidity	Date of emergence of fleas	Number of days in cocoon	Average	Remarks
12 July '12	50	* Lab. Cupboard	July 68.5 62.1 Aug. 63.3 56.3 Sept. 61.5 53.0	.79 .84 .86	2 27 July '12 8 2 Aug. '12 4 9 " 2 30 " +2 6 Sept. '12 1 30 " 1 20 Nov. '12 3 21 Dec. '12 4 4 Feb. '13 1 9 " 1 5 Mar. '13 3 8 " 1 25 " 10 emerged sub-sequently	15 21 28 49 56 65 116 147 192 197 221 224 241 say 250	At least 133	Remaining cocoons empty
15 "	50	Warm Cupboard	July 67.3 Aug. 64.5	.71 .71	1 26 July '12 5 30 " 6 3 Aug. '12 13 7 " 4 9 " 2 12 " 1 25 " 3 30 " 9 5 Sept. '12 2 9 Aug. '12 2 28 " 1 3 Sept. '12 2 6 " 6 17 Oct. '12 1 30 " 2 8 Nov. '12 2 4 Feb. '13 1 9 " 3 5 Mar. '13 3 emerged sub-sequently	11 15 19 23 25 28 41 46 52 25 44 50 53 94 107 116 204 209 233 say 240	29	Cocoons opened 5 Sept. contained 9 live fleas, 5 dead fleas, 1 dead larva; 2 dead fleas from 1 cocoon. Several cocoons spun back to back, in one of these double cocoons one compartment contained a dead flea and the other a live one
15 "	50	* Beehive	July 73.4 55.4 Aug. 64.9 45.6 Sept. 63.2 45.7	— —	2 9 Aug. '12 2 28 " 1 3 Sept. '12 2 6 " 6 17 Oct. '12 1 30 " 2 8 Nov. '12 2 4 Feb. '13 1 9 " 3 5 Mar. '13 3 emerged sub-sequently	129 25 44 50 53 94 107 116 204 209 233 say 240	129	Some cocoons opened 6 Sept. contained 2 living fleas Remaining cocoons opened 13 July '13 contained 1 dead larva, remainder empty

\* No record of temperature or humidity kept subsequently to Sept. 1912.

† Taken out of cocoons 6 Sept. '12.

NOTE. No accurate figures of mortality can be given.

TABLE XLI. *Cocoons C. gallinae. Eggs laid by adults fed on human blood; larvae reared and cocoons spun in Incubator 75 Wet; cocoons then removed to various situations.*

Date on which cocoons were placed under test conditions	No. of cocoons	Place to which cocoons were transferred	Temperature	Humidity	Date of emergence of fleas	No. of days in cocoon	Average	Mortality	Remarks
25 June '12	14	Incubator 75 Dry	June 75.8 July 76.2 Aug. 75.0	.54 .64 .56	8 8 July 3 13 " 1 24 Aug.	13 18 60	18	7 0/0	6 larvae emerged from cocoons, 5 pupated and fleas emerged, 1 failed to pupate Cocoons opened 5 Sept. and found to be empty, "false" cocoon
"	14	Incubator 93 Wet	95.9	.70	1 2 July	7	—	85 0/0	1 larva emerged from its cocoon but successfully pupated and emerged Remaining cocoons opened 4 Sept., 11 dead pupae, 1 dead larva, 1 empty "false" cocoon
28 June	14	Incubator 93 Dry	93.0	.55	2 8 July	10	—	(Total) 93 0/0 (In cocoon) 5 0/0	5 larvae emerged from their cocoons but pupated after a few days. Pupae subsequently dried up Remaining cocoons opened 4 Sept., 7 dead pupae, 1 dead larva
"	11	Cellar	max. min. June 56.5 55.6 July 60.1 59.3 Aug. 57.5 56.5	.93 .93 .93	4 26 July 1 3 Aug. 4 9 " 2 6 Sept.	28 36 42 70	41	Nil	2 larvae emerged from cocoons but pupated Remaining cocoons opened 6 Sept., 2 living fleas found
2 July	11	Beehive	July 73.4 55.4 Aug. 64.9 45.6 Sept. 63.2 45.7	— — —	1 15 July 4 26 " 1 2 Aug. 1 9 " 1 24 " 1 3 Sept. 1 6 "	13 24 31 38 53 63 66	36	(Total) 9 0/0 (In cocoon) Nil	1 larva emerged from its cocoon and died

TABLE XLII. *Cocoons L. musculi. Larvae taken from breeding cages, cocoons spun in Incubator 75 Wet*  
(Temperature 75° F., Humidity .85), and afterwards removed to various situations.

Date on which cocoons were placed under test conditions	No. of cocoons	Place to which cocoons were transferred	Temperature	Humidity	emergence of fleas	Date of emergence	No. of days in cocoon	Average	Mortality	Remarks
18 May '12	24	Incubator 75 Wet	May 75.6 June 74.9 July 75.0 Aug. 74.3	.86 .83 .86 .81	1 11 1 1	28 May 31 " 3 June 24 Aug.	10 13 16 98 104	24	37%	Remainder of cocoons opened 5 Sept., 1 dead flea, 6 dead pupae, 2 dead larvae
"	24	Incubator 93 Wet	May 95.1 June 94.5 July 95.2 Aug. 94.2	.75 .77 .74 .75	None emerged	None emerged	—	—	87% (at least)	Cocoons opened 4 Sept., 20 dead larvae, 1 dead pupa, 2 resting larvae (109 days) Resting larvae and remaining cocoon transferred to Incubator 75 Wet, both larvae died, cocoon a "false" one
"	24	Cellar	max. min. May 54.2 53.2 June 56.4 55.6 July 60.1 59.3	.93 .93 .93	7 8 3 1	18 June 27 " 5 July 15 "	31 40 48 58	38	Nil	2 cocoons opened 4 Sept., 2 living pupae, which subsequently died (109 days) Remaining cocoons opened and found empty, "false" cocoons
12 July	25	Incubator 75 Wet	75.0	.88	20 4	24 " 28 "	12 16	13	Nil	Remaining cocoon opened 5 Sept. contained living pupa (55 days)
"	25	Incubator 75 Dry	July 76.2 Aug. 75.0	.64 .56	1 12 6 3 2	19 " 24 " 27 " 30 " 7 Aug.	7 12 15 18 26	14	Nil	Remaining cocoon opened 5 Sept. empty, "false" cocoons
"	25	Incubator 93 Dry	July 93.0 Aug. 93.0	.57 .54	None emerged	None emerged	—	—	60% (at least)	Cocoons opened 4 Sept., 12 dead larvae, 2 dead pupae, 2 resting larvae, 1 shrunken larva but still fresh and moist, 1 empty 7 cocoons and 2 resting larvae transferred to Incubator 75 Wet, the larvae died and the cocoons proved to be empty (? "false")

"	25	Incubator 93 Wet	95.2	.74	None emerged	—	—	100 %	Cocoons opened 10 Sept., 14 dried up larvae, 6 dried up pupae, 5 dried up fleas
"	25	Cellar	max. min. July 60.1 59.3 Aug. 57.5 56.5 Sept. 56.0 54.3	.93 .93 .92	5 1 Aug. 11 9 " 3 13 1 24 " 1 6 Sept.	20 28 32 43 56	29	Nil	Remaining cocoons opened 6 Sept., 1 living flea, 1 living pupa, 3 empty, "false" cocoons (56 days)
"	25	Lab. Cupboard	July 68.5 62.1 Aug. 63.3 56.3	.79 .84	5 27 July 8 30 " 8 2 Aug. 3 9 "	15 18 21 28	19	Nil	Remaining cocoons opened 6 Sept., 1 resting larva (56 days)
"	25	Warm Cupboard	July 67.3 Aug. 64.5	.71 .71	7 26 July 5 30 " 5 3 Aug. 1 7 " 1 19 " 1 28 " 1 30 " 1 28 Sept.	14 18 22 26 38 47 49 78	24	Nil	Two cocoons opened 5 Sept., 10 empty, 1 living pupa, 1 resting larva (55 days), subsequently pupated and flea emerged 28 Sept., remaining cocoons "false"
"	25	Beehive	July 73.4 55.4 Aug. 64.9 45.6	— —	4 26 July 13 2 Aug. 4 9 " 1 12 "	14 21 28 31	21	Nil	Cocoons opened 6 Sept., 1 resting larva (56 days), 2 empty, "false" cocoons

TABLE XLIII. *Retarded emergence from cocoons C. fasciatus; full grown larvae taken from the breeding cages and placed with food to complete their development in Incubators 75 Wet and 85 Wet respectively.*

Date 1911	No. of larvae	Receptacle and food	Place	Date after which emergencies were noted	Temperature	Humidity	Number of days from date when full grown larvae were taken from the cages	
27 Jan. and 4 Mar.	120 to 150	Card Jar B.S. Rag	Incubator 75 Wet	May			2 fleas took	46
							4 "	51
							4 "	54
							4 "	58
							4 "	62
							7 "	65
							3 "	68
							4 "	71
							3 "	75
							1 "	83
17 Mar.	About 100	,,	,,	,,	Mar. 74.3	.76	1 "	49
					April 75.2	.78	2 "	52
					May 74.9	.85	5 "	55
					June 75.8	.86	1 "	56
					July 76.3	.85	11 "	59
					Aug. 75.0	.80	14 "	63
							13 "	66
							5 "	70
							5 "	74
							2 "	76
							1 "	88
							1 "	95
							1 "	102
							1 "	105
22 Mar.	,,	,,	,,	,,			1 "	131
							1 "	151
							2 "	56
							4 "	61
							2 "	69
8 Mar.	,,	,,	Incubator 85 Wet	,,			2 "	71
							3 "	117
					Mar. 83.7	.71	5 "	54
					April 84.0	.72	3 "	55
					May 84.0	.76	3 "	57
					June 84.0	.80	16 "	61
					July 85.1	.83	17 "	63
					Aug. 84.7	.81	10 "	65
							6 "	68
							6 "	71
							5 "	73
							5 "	75
							1 "	77
							1 "	82
							1 "	84
							1 "	86
							1 "	90
							1 "	92
							1 "	108
							2 "	114
							1 "	121
							1 "	127
							3 "	131
							1 "	141
							1 "	149

ii. *Influence of immersion in water upon the vitality of Cocoons. Ct. canis* (Table XLIV).

An attempt was made to ascertain what powers, if any, fleas might possess of surviving inundation. The cocoon stage seems to afford the only chance of fleas being able to survive actual flooding of their habitat. A plentiful supply of the cocoons of *Ct. canis* induced the selection of this species for a trial. The larvae were allowed to spin at the bottom of card jars and, after allowing a few days for the silk to harden, the loose sand was emptied out and water poured in until the cocoons were thoroughly submerged.

The water was poured off from the various jars after periods of half an hour, three quarters of an hour, one hour, 12 hours and one week; they were then placed to dry and the fleas allowed to emerge in the laboratory cupboard. As the trial took place during August and September 1911, emergence would be in response to natural conditions of heat and moisture apart from the wetting.

It will be seen that the cocoons of *Ct. canis* easily withstood 12 hours' soaking but were destroyed by a week's submergence. Too much reliance must not be placed on the difference in the number of emergencies in the different experiments as the numbers put in were not accurately counted, but only roughly estimated by dividing a large batch of larvae.

TABLE XLIV. *Immersion Test. Cocoons Ct. canis.*

Date 1911	Approximate number	Period under water	Emergence
1 Aug.	100	half-an-hour	98 fleas on 17 Aug. 2 „ 30 „ 5 „ 28 Sept.
1 „	100	three-quarters of an hour	80 „ 17 Aug. 12 „ 30 „ 1 „ 28 Sept.
1 „	100	one hour	66 „ 15 Aug. 15 „ 30 „ 6 „ 28 Sept.
1 „	100	12 hours	62 „ 17 Aug. 1 „ 30 „ 3 „ 28 Sept.
10 „	100	one week	No emergence; two opened contained 1 dried up flea, 1 dried up pupa

iii. *Influence of external conditions at different periods of larval existence upon the duration of the cocoon stage C. fasciatus.*

Experiments were undertaken with a view to discover if the length of the resting period of the larvae of *C. fasciatus* within the cocoon was influenced by changes in the conditions of heat and humidity during the active period of larval life, and also to ascertain if possible at what period of growth the larvae could be most readily influenced.

Two batches of larvae were sorted out from a cage, one judged to be about half grown and the other ready to spin their cocoons. Both lots were subjected to similar treatment but otherwise kept apart, they are referred to as "full grown" and "half grown" respectively. These batches were subdivided, one half of each batch being allowed to feed and spin in incubator 75 Wet while the other half completed its development to the cocoon stage in receptacles kept in the cage from which they had been taken.

In both series of experiments the cocoons as formed were further separated into two batches, one batch of each lot being transferred to incubator 93 Dry and the other being allowed to remain where the larvae had spun. There are therefore eight records to follow up, viz. four batches in incubator 93 Dry, consisting of cocoons from "half grown" larvae spun in the cage and incubator 75 Wet and from "full grown" spun in the cage and incubator 75 Wet: two batches, one from "half grown" and one from "full grown" larvae maintained in incubator 75 Wet throughout and a similar pair kept in the cage.

The drastic conditions prevailing in incubator 93 Dry produced such a high mortality that the experiment more or less failed of its original purpose and a second series of trials were started on the same lines with the exception that incubator 93 Wet was substituted for 93 Dry.

A conclusion which may apparently be gleaned from the results of the first series is that the transference of "half grown" larvae from the cages to incubator 75 Wet is an unfavourable preparation for a higher temperature and drier atmosphere in the cocoon stage (compare batches B and D). I think further it may be taken as settled that a rise in temperature during active larval life, such as removal from the cage to incubator 75 Wet, does not act as a stimulus to lengthen the cocoon period. In fact it seems that the moderate conditions in incubator 75 Wet induce the individuals to curtail their resting period and to develop and emerge after a comparatively



short rest. Hot dry conditions appear to inhibit the development of individuals predisposed to a long rest but are not necessarily fatal, provided a reduction in temperature occurs within three or four months. The cage conditions on the other hand allow full scope for any natural predisposition of the species for a long cocoon period.

It is possible that extremes of climate may only control the prevalence of the adults of this species by rendering a distributed emergence impossible and inducing a condensed periodic one in its place.

The results of the second series, while fully in accord with the above conclusions, tend to support the phenomena brought to light in the monthly cocoon tests with this species, Table XXXIII; that this species has a fluctuating, or periodic, constitution as regards its powers of endurance. Allowance must however be made for the difference in incubator conditions in winter and summer brought to light in the newly hatched larvae trials (see footnote on p. 459 in reference to humidity conditions in incubators).

FIRST SERIES. "*Half grown*" larvae of *C. fasciatus* taken from cage and allowed to finish feeding and spin their cocoons in Incubator 75 Wet (temperature about 75° F., humidity about 70).

NOTE. The numbers giving the days spent in the cocoon do not increase in exact sequence in all cases owing to the fact that the cocoons were transferred in batches and the fleas as they emerged were presumed to have come from the earliest available batch.

Batch A left in Incubator 75 Wet. Temperature about 75° F.

Humidity about 65 to 70.

Date	Number of cocoons	Emergence of fleas	Approximate number of days in cocoon	Number of days in cocoon calculated from average date given in col. 1
22—29 Nov. '11	27	2 on 28 Nov.	6	4
(average date		2 1 Dec.	9	7
24 Nov.)		8 5 „	13	11
		9 11 „	{ 6 16	17
			{ 3 19	
		2 12 „	17	18
		1 11 Jan.	47	48
		1 23 „	55	60

2 cocoons opened 4 July '12 found empty ("false" cocoons).

Batch B transferred to Incubator 93 Dry. Temperature about 93° F.

Humidity about 56 to 60.

Date	Number of cocoons	Emergence of fleas	Number of days in cocoon
22—29 Nov. '11	27	(None)	—

On the 16 March '12, 19 cocoons were opened and found to contain 13 dead fleas, 5 dead larvae, 1 resting larva.

The remaining cocoons and 1 resting larva were transferred to Incubator 75 Wet; the larva died.

On the 8 July '12, all these cocoons were opened and found to contain dried up larvae.

FIRST SERIES. "*Half grown*" larvae of *C. fasciatus* taken from cage and allowed to finish feeding and to spin cocoons in the cage.

Batch C left in Cage.

Date	Number of cocoons	Emergence of fleas	Approximate number of days in cocoon	Number of days in cocoon calculated from average date given in col. 1
13 Dec. '11—	30	1 on 9 Jan. '12	say 27	16
19 Jan. '12		3 8 Feb.	„ 57	46
(average date		1 26 March	„ 103	92
24 Dec.)		1 1 April	„ 109	98
		1 23 „	„ 131	120
		1 27 „	„ 135	124
		3 20 June	„ 189	178
		4 1 July	„ 191	189
		1 3 „	„ 190	191
		1 10 Aug.	„ 221	229
		1 13 „	„ 224	232
		3 9 Sept.	„ 251	259
		1 15 „	„ 257	265

Remaining cocoons opened 25 July '13, all were empty except two which contained living larvae that had been resting for a period of from 563 to 590 days.

Batch D transferred to Incubator 93 Dry. Temperature about 93° F.

Humidity about .56 to .60.

Date	Number of cocoons	Emergence of fleas	Number of days in cocoon
13 Dec. '11—9 Jan. '12	30	1 on 20 Dec. '11	say 7

On 16 March '12, after a period of about 76 days, 6 cocoons were opened and found to contain 1 dead flea, 1 dead larva, 4 resting larvae.

The remaining cocoons and resting larvae were transferred to Incubator 75 Wet and there emerged

			Total number of days in cocoon including those spent in incubator 93 Dry
2 fleas on 3 April	18 days at 75° F., Humidity .79		94
1 flea 6 „	21 „ „		97
2 fleas 8 „	23 „ „		99
1 flea 11 „	26 „ „		102
1 „ 19 „	34 „ „		114

Remaining cocoons opened 3 July '12 contained 5 dried up fleas, 1 dried up larva, 14 empty cocoons ("false" cocoons<sup>1</sup>).

<sup>1</sup> The large number of "false" cocoons in these high temperature trials is accounted for by the larvae emerging and dying outside the cocoons where they become brittle and break into unrecognisable fragments.

FIRST SERIES. "*Full grown*" larvae of *C. fasciatus* taken from cage and allowed to finish feeding and to spin their cocoons in Incubator 75 Wet (temperature about 75° F., humidity about 70).

Batch A left in Incubator 75 Wet. Temperature about 75° F. Humidity about 65 to 70.

Date	Number of cocoons	Emergence of fleas	Number of days in cocoon
18 Nov. '11	31	6 on 28 Nov. '11	10
		4 1 Dec. '11	13
		1 5 "	17
		2 16 "	28
		2 1 Jan. '12	44
		2 4 "	47
		1 6 "	49
		2 8 "	51
		4 9 "	52
		1 11 "	54
		1 12 "	55
		2 15 "	58
		1 23 "	66
		2 26 "	69

Batch B transferred to Incubator 93 Dry. Temperature about 93° F.  
Humidity about 56 to 60.

Date	Number of cocoons	Emergence of fleas	Number of days in cocoon
18 Nov. '11	31	3 on 25 Nov. '11	7
		1 1 Dec. '11	13

On the 16 March '12, after 119 days in Incubator 93 Dry, 6 cocoons were opened and found to contain 3 dead fleas, 3 resting larvae.

The resting larvae and balance of the cocoons were transferred to Incubator 75 Wet and there emerged

				Total no. of days in cocoon including 119 spent in Incubator 93 Dry
3 fleas on 1 April '12	3	16 days at 75° F., Humidity about 80		135
6 " 3 "	6	18 " " "		137
4 " 6 "	4	21 " " "		140
1 flea on 8 "	1	23 " " "		142

Cocoons opened 3 July '12, 2 dried up fleas, 2 dried up pupae, 4 dried up larvae.

FIRST SERIES. "*Full grown*" larvae of *C. fasciatus* taken from cage and allowed to finish feeding and to spin in the breeding cage.

Batch C left in Cage.				
Date	No. of cocoons	Emergence of fleas	Approximate no. of days in cocoon	No. of days in cocoon calculated from average date given in col. 1
19 Nov.—	32	1 on 28 Nov. '11	say 9	3
1 Dec. '11		1 20 Dec. '11	„ 31	25
(average date		1 13 Jan. '12	„ 55	49
25 Nov.)		2 23 April '12	„ 144	150
		1 27 „	„ 148	154
		1 24 May '12	„ 175	184
		2 20 June '12	„ 202	208
		1 1 July '12	„ 213	219
		1 3 „	„ 215	221
		3 26 „	„ 238	244
		1 8 Aug. '12	„ 251	257
		3 9 Sept. '12	„ 283	289
		4 15 „	„ 289	299
		1 19 Jan. '13	„ 415	421
3 fleas, date of emergence unrecorded, found dead 25 July '13				

Remaining cocoons opened 25 July '13 contained 1 dead and 3 living larvae; these latter had "rested" from 571 to 613 days.

Batch D transferred to Incubator 93 Dry. Temperature about 93° F.  
Humidity about .56 to .60.

Date	Number of cocoons	Emergence of fleas	Number of days in cocoons
19 Nov.—1 Dec. '11	32	None	—

On 16 March '12, after about 112 days in Incubator 93 Dry, 3 cocoons were opened and found to contain resting larvae.

The resting larvae and remaining cocoons were transferred to Incubator 75 Wet and there emerged

				Total no. of days in cocoon including those (112 approximately) spent in Incubator 93 Dry
3 fleas on 1 April	16 days at 75° F., Humidity .80 to .86			128
3 „ 3 „	18 „ „ „			130
2 „ 6 „	21 „ „ „			133
2 „ 8 „	23 „ „ „			135
1 flea on 11 „	26 „ „ „			138
3 fleas on 16 „	31 „ „ „			143
1 flea on 19 „	34 „ „ „			146
1 „ 24 „	39 „ „ „			151
1 „ 16 June	92 „ „ „			204

Remaining cocoons opened 3 July '12 contained 2 dried up fleas, 1 dried up pupa, 3 dried up larvae, 9 "false" cocoons.

SECOND SERIES. "*Half grown*" larvae of *C. fasciatus* taken from breeding cage and allowed to finish feeding and to spin cocoons in Incubator 75 Wet.

Batch A left in Incubator 75 Wet. Temperature about 75° F. Humidity about .80 to .86.

Date	No. of cocoons	Emergence of fleas	Approximate no. of days in cocoon	No. of days in cocoon calculated from average date given in col. 1
18—26 March '12	31	2 on 1 April '12	say 14	10
(average date 22 March)		6 3 "	" 16	12
		3 8 "	" 20	17
		5 11 "	" 20	20
		1 16 "	" 25	25
		4 23 "	" 32	32
		1 27 "	" 36	36
		1 30 "	" 35	39
		1 6 May '12	" 41	45
		2 16 "	" 51	55
		2 21 "	" 56	60
		1 24 "	" 59	63
		1 28 "	" 63	67

Cocoons opened 4 July '12, 1 "false" cocoon.

Batch B transferred to Incubator 93 Wet. Temperature about 93° F. Humidity about .67.

Date	Number of cocoons	Emergence of fleas	No. of days in cocoon
18—26 March '12	32	2 on 28 March '12	say 10

Cocoons opened 2 July '12 contained 22 dried up fleas, 7 dried up larvae, 1 "false" cocoon.

SECOND SERIES. "*Half grown*" larvae of *C. fasciatus* allowed to finish feeding and to spin cocoons in the breeding cage.

Batch C left in Cage.

Date	No. of cocoons	Emergence of fleas	Approximate no. of days in cocoon	No. of days in cocoon calculated from average date given in col. 1
18 April—	18	4 on 24 May	say 36	30
8 May '12	2	20 June	" 63	57
(average date 24 April)	1	3 July	" 76	70
			Cocoon opened and living flea emerged	
	2	10 Aug.	" 114	118
	2	13 "	" 117	121
	1	1 Sept.	" 127	130
	*2	9 "	" 135	138
			Cocoons opened and living fleas emerged	

10 cocoons opened 9 Sept. '12 contained 1 dead flea, 2 living fleas, 2 dead larvae, 1 living larva which subsequently died.

*Bionomics of Fleas*

Batch D transferred to Incubator 93 Wet. Temperature about 93 to 95° F.

Humidity about .70 to .75.

Date	Number of cocoons	Emergence of fleas	No. of days in cocoon
18 April—2 May '12	18	none	—

Cocoons opened 2 July '12 contained 2 dried up fleas, 4 dried up pupae, 4 resting larvae.

The resting larvae were transferred to Incubator 75 Wet to see if they would continue their development, they died without becoming pupae.

SECOND SERIES. "*Full grown*" larvae of *C. fasciatus* taken from breeding cage and allowed to spin cocoons in Incubator 75 Wet.

Batch A left in Incubator 75 Wet. Temperature about 75° F. Humidity about .80 to .85.

Date	No. of cocoons	Emergence of fleas	Approximate no. of days in cocoon	No. of days in cocoon calculated from average date given in col. 1
14—19 March '12	33	1 on 22 March	say 8	6
(average date		9 26 "	" 12	10
16 March)		6 29 "	" 15	13
		4 1 April	" 18	16
		2 21 May	" 63	66
		1 24 "	" 66	69
		1 3 June	" 76	79
		1 8 "	" 81	85

Cocoons opened 4 July '12 contained 5 dried up larvae.

Batch B transferred to Incubator 93 Wet. Temperature about 93° F.

Humidity about .67 to .70.

Date	No. of cocoons	Emergence of fleas	Approximate no. of days in cocoon	No. of days in cocoon calculated from average date given in col. 1
14—19 March '12	33	1 on 22 March	say 8	6
(average date		6 26 "	" 12	10
16 March)		1 28 "	" 14	12
		2 1 April	" 17	16
		1 8 "	" 24	23
		2 12 "	" 28	27

Cocoons opened 2 July '12 contained 7 dried up fleas, 11 dried up larvae.

SECOND SERIES. "*Full grown*" larvae of *C. fasciatus* allowed to spin their cocoons in the breeding cage.

Batch C left in Cage.

Date	No. of cocoons	Emergence of fleas	Approximate no. of days in cocoon	No. of days in cocoon calculated from average date given in col. 1
19 March—	21	2 on 23 April	say 35	28
8 April '12	3	27 "	" 39	32
(average date	2	2 May	" 38	37
26 March)	1	1 July	" 98	97
	1	3 "	" 100 Cocoon opened	99
	1	8 Aug.	" 136	135
	1	9 Sept.	" 168	167
	1	10 Oct.	" 199	198
	2	26 "	" 213	214

Remaining cocoons opened 25 July '13, one contained a living larva which had been resting between 473 and 493 days, remaining cocoons empty.

Batch D transferred to Incubator 93 Wet. Temperature about 93° F.  
Humidity about .70 to .75.

Date	Number of cocoons	Emergence of fleas	Approximate no. of days in cocoon
19 March—8 April '12	21	1 on 18 April	say 30
		1     5 Aug.	,, 96

Cocoons opened 2 July '12 contained 6 dried up fleas, 4 dried up pupae, 9 dried up larvae, 1 resting larva. The resting larva was transferred to Incubator 75 Wet, 2 July. This larva pupated on 30 July. A flea emerged on 5 Aug., say 90 days in Incubator 93 Wet. This individual therefore continued resting without a cocoon in Incubator 75 Wet for 28 days and emerged as adult in 6 days. Total 96 days.

iv. *Significance of "Hard" and "Soft" cocoons of C. fasciatus.*

Experiments were made in order to ascertain whether those individuals that formed hard cocoons deferred emergence for a longer period than those whose cocoons were of the softer type. Two sets of experiments were performed, one in which sand was used and one in which house dust replaced the sand to test whether the nature of the attached fragments had any influence upon the character of the cocoon.

When the larvae of *C. fasciatus* are given finely sifted house dust (sweepings of floors) in place of silver sand the silk of their cocoons differs from that of those formed in sand. The cocoons are tougher and the silk is whiter and more papery in appearance, and is less suggestive of thin glue that has been allowed to harden, than is the case when grains of sand are embedded in place of dust.

It seems possible that this variation is in part, perhaps largely, due to the small size of the particles of dust and their more absorbent nature producing a different action in the setting of the silk. Sand is composed of larger particles which are individually non-absorbent, and this no doubt delays the drying and affords an opportunity for the threads of mucilage to coalesce into larger masses before drying takes place<sup>1</sup>.

<sup>1</sup> My assistant Mr J. H. Turner states that he has noticed that the "hard" cocoons generally have smaller grains of sand attached than the "soft"; presumably such a difference must rest on a selective act on the part of the larvae when spinning their cocoons. There is also a well marked difference between the individual cocoons of *C. fasciatus* apart from the material worked into them, and although I have not opened sufficient examples to make any definite generalisation, the cocoons of the different species show marked divergencies when all are spun in sand. Cocoons of *X. cheopis* are softer as well as tougher than those of *C. fasciatus*, approximating towards the type of *P. irritans* which is composed of a felt of actual silk threads with the extraneous matter attached on the outer side.

A number of larvae of *C. fasciatus* were taken from the cages and allowed to spin in incubator 75 Wet, in the case of one series in sand and of the other in dust. The stronger cocoons among those spun in dust are more correctly described as "firm" than "hard"; for the sake of uniformity, however, the latter term has been used in both series of experiments. The question as to whether an individual cocoon should be regarded as "soft" or "hard" was decided by pressing upon the cocoon with the side of a fine needle, near the point. Those which yielded were designated as "soft," those which resisted as "hard." That the stage of development of the insect has nothing to do with the consistence was shown by the fact that some of the resistant cocoons were found to be empty. When covered with sand the hardness was more extreme, quite considerable pressure being required to crush empty ones.

The cocoons were allowed to remain in incubator 75 Wet for a few days after spinning and were then sorted out into batches of "hard" and "soft" respectively and then transferred to incubator 93 Dry. In the sand series some cocoons of each class were kept in incubator 75 Wet as a control. A few examples of those spun in dust, opened to test the reliability of the sorting, gave the following particulars:

8 "soft" showed	9 "hard" showed
1 pupa	3 pupae
1 resting larva	3 resting larvae
6 empty (fleas emerged)	3 empty (fleas emerged)

While the results obtained support the view that the larvae in "hard" cocoons are better able to resist conditions of heat and drought than those in the "soft," it is not clear that the *solidity* of the cocoon is the only factor. It will be noticed that there was a higher mortality among the "soft" than the "hard" in incubator 75 Wet, sand series, which suggests that "hard" cocoons may be associated with individuals of greater vitality and that the question is not merely one of the type of cocoon or a special adaptation for resting. It is, however, true that the resting stage is longer in the hard cocoons, which favours the theory that the greater solidity of these cocoons diminishes the risks of a deferred emergence.

These experiments also bring into special prominence the inhibitory action of incubator 93 Dry with regard to emergence, which offers a contrast to the results obtained in incubator 75 Wet, where the average duration of the cocoon period is very short in comparison.



*"Hard" and "Soft" Cocoons C. fasciatus.*

*Cocoons spun in dust. Larvae taken from cages and allowed to spin their cocoons in finely sifted house dust in Incubator 75 Wet and then transferred to Incubator 93 Dry (humidity about .56 to .60).*

*"Soft" batch.*

Date	Number	Fleas emerged	No. of days in cocoon
6 Nov. '11	22	5 on 21 Nov.	15
		7 25 "	19

On 16 March '12 after 131 days in Incubator 93 Dry, 2 cocoons were opened and 1 resting larva, 1 empty ("false" cocoon), were found.

The remaining cocoons and the resting larva were transferred to Incubator 75 Wet. The larva died. Fleas emerged from cocoons as under :

				Total no. of days in cocoon, including 131 days in Incubator 93 Dry
1 flea emerged 1 April	16 days at 75° F., Humidity .79			147
" " 16 "	31 " " "			162
" " 23 "	38 " " "			169

On 4 July '12 remaining cocoons opened, 5 dried up larvae found.

*"Hard" batch.*

Date	Number	Fleas emerged	No. of days in cocoon
6 Nov. '11	29	6 on 24 Nov.	18
		1 27 "	21

On 16 March '12, 2 cocoons were opened, both contained resting larvae; these together with the remaining cocoons were transferred to Incubator 75 Wet and there emerged

				Total no. of days in cocoon including 131 days in Incubator 93 Dry
2 fleas emerged 1 April	16 days at 75° F., Humidity .79			147
8 " " 6 "	21 " " "			152
1 flea " 8 "	23 " " "			154
2 fleas " 11 "	26 " " "			157
1 flea " 30 "	45 " " "			176

On 4 July cocoons opened, contained 3 dried up fleas, 3 dried up larvae.

From a few of the unsorted cocoons which were left in Incubator 75 Wet, when the others were transferred to Incubator 93 Dry, fleas emerged as follows :

Date	Number	Fleas emerged	No. of days in cocoon
6 Nov. '11	Number unknown	1 on 21 Dec. '11	45
		2 24 "	48
		1 12 Jan. '12	67
		1 16 "	71
		4 18 "	73

*"Hard" and "Soft" Cocoons C. fasciatus.*

*Cocoons spun in sand. Larvae taken from cages and allowed to spin their cocoons in fine silver sand in Incubator 75 Wet and then transferred to Incubator 93 Dry (humidity about .56 to .60).*

*"Soft" batch.*

Date	Number	Fleas emerged	No. of days in cocoon
5 Nov. '11	24	1 on 16 Nov. '11	11

On 16 March '12, 8 cocoons were opened and found to contain 1 dead flea, 4 dead larvae, 3 resting larvae.

The resting larvae and the remaining cocoons were transferred to Incubator 75 Wet. (Two of the larvae died, one pupated and a flea duly emerged.)

4 fleas emerged 6 April, 132 days at 93° F., Humidity .56 to .60; 21 days at 75° F., Humidity .79. Total 153 days.

Remaining cocoons opened 3 July '12 contained 3 dried up fleas, 6 dried up larvae, 2 "false" cocoons.

*"Hard" batch.*

Date	Number	Fleas emerged	No. of days in cocoon
5 Nov. '11	39	none	—

On 16 March '12, after 132 days in Incubator 93 Dry, 11 cocoons were opened and found to contain 2 dead fleas, 7 larvae somewhat shrunken and devoid of movement but not discoloured or dried, 2 resting larvae which subsequently pupated and produced adults.

The resting larvae and remaining cocoons were transferred to Incubator 75 Wet.

			Total number of days in cocoon including 132 days in Incubator 93 Dry
2 fleas emerged 3 April	18 days at 75° F., Humidity .79		150
2 " 6 "	21 " " "		153
4 " 8 "	23 " " "		155
2 " 11 "	26 " " "		158
1 flea emerged 16 "	31 " " "		163
1 " 27 "	42 " " "		174

Cocoons opened 3 July '12 contained 3 dried up fleas, 7 dried up larvae<sup>1</sup>.

*"Hard" and "Soft" Cocoons C. fasciatus.*

*Cocoons spun in sand. Larvae taken from cages and allowed to spin their cocoons in fine silver sand in Incubator 75 Wet and left under these conditions for the fleas to emerge (remainder of batch of cocoons used for experiments in previous table).*

*"Soft" batch.*

Date	Number	Fleas emerged	No. of days in cocoon
5 Nov. '11	24	1 on 13 Nov. '11	8
		1 16 "	11
		4 18 "	13
		2 19 Dec. '11	44
		3 24 "	49
		1 1 Jan. '12	57

Cocoons opened 3 July '12 contained 8 dried up larvae<sup>1</sup>.

<sup>1</sup> The record for "hard" and "soft" cocoons spun in sand is incomplete, no record was made on July 3rd 1912 of the number of empty or "false" cocoons in the "hard" batch in Incubator 93 Dry or in the "soft" batch in Incubator 75 Wet.

"Hard" batch.

Date	Number	Fleas emerged	No. of days in cocoon
5 Nov. '11	39	1 on 13 Nov. '11	8
		1 16 "	11
		2 25 "	20
		1 19 Dec. '11	44
		5 22 "	47
		2 23 "	48
		2 24 "	49
		2 26 "	51
		3 27 "	52
		3 28 "	53
		4 1 Jan. '12	57
		2 4 "	60
		2 8 "	64
		1 9 "	65
		2 11 "	67
		1 29 "	85
		1 11 Feb. '12	98

Cocoons opened 3 $\frac{1}{2}$  July '12, 4 empty, "false" cocoons.

4. ADULTS (Tables XLV to LIX).

i. *Duration of Life unfed.*

*P. irritans* (Tables XLV and L). The experiments with adult specimens of *P. irritans* just emerged from their cocoons and unfed are too few to be conclusive; the results obtained suggest, however, that the species cannot survive long without feeding if exposed to warmth or heat, even if the conditions are humid. In cool and moist places, however, a life of more than 100 days was recorded (see Table XLV). Allowing them to burrow free in sand does not appear to favour an extended life.

*C. fasciatus* (Tables XLVI and L) when kept in paper tubes, cannot compete with *P. irritans* as regards its power of surviving without food. When kept in a paper tube no life of more than 31 days was recorded even in the most favourable situation; but, as already noted in the general remarks on life history, when allowed to burrow in sand an individual survived starvation for 95 days. In less favourable situations there is not much to choose between the species.

*X. cheopis* (Tables XLVII and L). It does not appear that adults of *X. cheopis* differ very much from *C. fasciatus* in their power of enduring starvation. It is possible that they stand hot and dry conditions a little better, but, to judge from the beehive record, they are quite unsuited for a cold situation. Burrowing in sand would appear to increase their powers of resistance.

*Ct. canis* (Tables XLVIII and L). This species has powers of endurance about equal to those of *C. fasciatus* when tested by the paper tube method; burrowing free in sand does not seem to appreciably extend its powers of survival unfed.

*C. gallinae* (Tables XLIX and L). Only a small number were available and these were used for tests in cool situations only. When tested by the paper tube method they show endurance on about the same scale as *C. fasciatus* or *X. cheopis*, but burrowing in sand would appear to make a vast difference to them, one specimen surviving 127 days and showing an endurance equalled only by *P. irritans*.

ii. *Duration of Life when fed.*

(a) *for an initial period only.* *P. irritans* and *C. fasciatus* (Tables LI and LII). Newly emerged specimens of these two species were fed for certain periods and then tested as in the previous series. It will be seen from a comparison of Tables XLV and XLVI with LI and LII respectively that, under cool conditions with high humidity, the fact of a flea having previously fed makes little material difference to its powers of endurance, but that under warm to high temperatures with a low humidity, a meal to start with is a very valuable asset.

For example, in case of *P. irritans*, in the cellar, the longest length of life when starved subsequent to a period of feeding was 121 days, while, in case of adults starved from emergence, a life of 126 days was recorded. In incubators 75 Dry and 85 Dry, on the other hand, previous feeding raised the figure obtained for length of life from 5 to 17 and 5 to 12 days respectively.

It is greatly to be regretted that only single individuals of *P. irritans* were available for this test. The consistent increase in length of life recorded as we pass from hot and dry to cool and moist situations may, however, be taken as confirmatory of the trustworthiness of the results.

*C. fasciatus*. This species, like *P. irritans*, does not gain by feeding prior to starvation; feeding for several days tends to lessen, not increase, their powers of survival and leaves the average total length of life practically unaltered.

*Ct. canis* (Table LIII). The small trial made with this species cannot be compared with the *P. irritans* and *C. fasciatus* tests, as the specimens were of unknown age, and had already been 24 hours in incubator 75 Wet before the test was made.

(b) *At regular intervals.* *P. irritans.* When fed on man daily, individual specimens of this species have lived for various periods up to 513 days (see Table LIV (c)), and, when fed weekly, also over a year if kept under cool humid conditions (see Table LIV (d)).

It will be seen from Table LIV that the summer is just as favourable as the winter. Several of the early deaths recorded for this species were due to accident, becoming gummed to the glass bottom of the box by their own dejecta or losing portions of the legs. On a few occasions, deaths have been quite inexplicable; in one instance some six or eight freshly emerged specimens all died within 10 or 12 days, although afforded equal opportunities of feeding with batches that enjoyed extended lives.

Buckland records (*Curiosities of Natural History*, fourth series, p. 123) on the authority of the owner of some performing fleas, a specimen which lived for eighteen months—a period which slightly exceeds the longest life recorded in the present experiments.

*C. fasciatus.* The number and variety of other experiments on hand prevented the carrying through of cage experiments with this species and *X. cheopis* to test the length of life of these species when free upon their natural hosts. Two or three early attempts failed, owing to paucity of supplies, and it has not been found possible to repeat them. Experiments in which the fleas were fed on rats by the box method, point to the necessity of cool, or at least moderate conditions of temperature (see Table LV, under date of 12th October, 1910, in incubator 85° Wet) and, when not living in close association with the rat, to the need of more frequent chances of feeding than were found necessary for *P. irritans*. The longest life recorded is 106 days, and this is probably nearer to the correct length of time than the average lives—the early deaths which bring down the average being probably those of individuals which never commenced feeding.

iii. *Duration of Life if fed on an alien host.*

*C. fasciatus* fed on man (Table LVI). When fed on man the average length of life is, on the whole, higher than when fed on rats by the box method, possibly because the feeding being conducted at night while in bed the time was apt to be extended longer than the usual 15 minutes. The 10 deaths in 13 days, recorded in the experiment under date 15th July, 1911, were due to a misadventure and should not be considered when judging results.

*X. cheopis.* The experiment (Table LVI) shows this species to be better adapted to a human host than *C. fasciatus*, lives of over 70 days

being frequent. Observation of the specimens during the course of the experiment provides confirmatory evidence of this, in that they were seen to be very lively and to be feeding vigorously, judging by the full state of their stomachs.

*Ct. canis* and *Ct. felis*. It will be seen from Table LVII that these two species can exist quite successfully on a diet of human blood—the lives recorded being of considerable extent, as long as 345 and 185 days respectively.

*C. gallinae* (Table LVII) is also able to live for a considerable period when fed on man. The single specimen captured on the dog lived 224 days after capture.

*L. musculi* (Table LVII). A certain percentage of individuals of this species will certainly feed on man, the blood was quite visible in previously unfed specimens, but they do not bite freely, as do *X. cheopis* and *C. fasciatus*. Life was only of very short duration and did not exceed 10 days in case of any of the fleas tested.

*Spilopsyllus cuniculi* (Table LVII). This species feeds much more freely on man than does *L. musculi*; I think all the specimens tried bit, but they are not transparent enough to show blood in their stomachs. As will be seen from the table, their record is consistent with this view.

Attempts to feed *P. irritans* and *Ct. canis* on rats (Table LV) show that the last named species will certainly feed, but, as regards the former, it is somewhat doubtful whether the insects did more than experiment, the lives of those fed upon rats by the box method being no longer than one would expect if kept unfed under the same circumstances.

iv. *Attempts to induce fleas to feed upon material other than the blood of a living animal.*

The experiments in which fleas were given the opportunity of sucking water or feeding on syrup, broth, etc., have not been successful in establishing as a fact whether they do feed, or whether they only obtain a benefit by the moisture in the air obtained by a pad of blotting paper moistened being placed in the box with them. The fleas were observed to jump on the pad and sometimes put down their heads as if to feed, but as the process was only followed with a hand lens and the fleas were kept in a box it is not possible to say whether suction actually took place.

A number of dissections was made of individuals kept under similar conditions to those mentioned above; carmine and other stains,

that were successfully used with larvae of fleas and of flies, were mixed with the artificial foods or water, but no trace of the stain was ever found in the gut of the adult flea.

TABLE XLV. *Adult fleas. P. irritans. Length of life unfed. Fleas freshly emerged from cocoons, placed in filter paper tubes and buried in sand in various situations.*

Date	No.	Place	Temperature		Humidity	Detail
12 May '11	1	Incubator 85 Wet	83·8		·81	dead in 11 days
12 „	1	Incubator 75 Wet	75·1		·91	„ 9 „
10 „	1	Incubator 85 Dry	84·0		·61	„ 5 „
10 „	1	Incubator 75 Dry	74·7		·53	„ 5 „
15 „	1	Warm Cupboard	63·5		·65	„ 6 „
			max.	min.		
15 „	1	Lab. Cupboard	62·6	54·1	·81	„ 11 „
4 Aug. '10	2	„	74·4	66·0	·74	{1 „ 9 „ 1 „ 10 „
12 Dec. '10	1	Damp corner of Cellar	47·3	46·0	over ·94	„ 71 „
1 Mar. '11	1	„	51·8	50·3	„ ·93	dead in 126—130 days
27 April '11	2	„	57·6	56·3	„ ·93	{1 dead in 63 days 1 „ 91 „
18 Aug. '11	1	„	62·9	61·6	„ ·94	escaped after 1 month
19 „	3	„	62·2	61·3	„ ·94	{1 dead in 28 days 2 „ 32 „
28 April '11	2	Beehive	69·5	43·1	—	{1 „ 15 „ 1 „ 20 „

TABLE XLVI. *Adult fleas. C. fasciatus. Length of life unfed. (a) Fleas freshly emerged from cocoons, placed in filter paper tubes and replaced in soil pots in the situations in which they had been reared from cocoons.*

Dates between which the fleas were obtained	No.	Place	Temperature	Humidity	Detail	Longest period of survival, days	Average period of survival, days
17 Aug. '10—	18	Incubator	Dry 84·5	·71	2 dead in 4 days	13	8
28 Sept. '10		85 Wet			1 „ 6 „		
					8 „ 7 „		
					1 „ 8 „		
					1 „ 9 „		
					1 „ 11 „		
					1 „ 12 „		
					2 „ 13 „		
					1 lost		

TABLE XLVI.—*Continued.*

Dates between which the fleas were obtained	No.	Place	Temperature	Humidity	Detail	Longest period of survival, days	Average period of survival, days
15 Aug. '10—	13	Incubator	Dry 74·3	·65	4 dead in 5 days	7	6
29 „		75 Wet			3 „ 6 „		
					6 „ 7 „		
19 Aug. '10—	17	Laboratory	estimate 60·0	·80	2 „ 5 „	15	10
3 Oct. '10		Cupboard			1 „ 6 „		
					7 „ 10 „		
					1 „ 11 „		
					1 „ 12 „		
					2 „ 13 „		
					2 „ 14 „		
					1 „ 15 „		
25 Aug. '10—	13	Cellar	max. min. 58·3 57·3	estimate ·90	2 „ 7 „	17	14
6 Oct. '10					1 „ 10 „		
					2 „ 12 „		
					1 „ 13 „		
					1 „ 15 „		
					1 „ 16 „		
					5 „ 17 „		
1—7 Dec. '10	1	„	48·7 47·7	·93	1 „ 6 „	—	—

(b) *Fleas taken from stock reared in Incubator 75 Wet.*

28 Dec. '10—	12	Cellar	46·5 45·3	·93	2 „ 17 „	31	24
28 Jan. '11					4 „ 21 „		
					4 „ 28 „		
					2 „ 31 „		
28 Dec. '10—	7	Laboratory	53·5 43·2	·85	1 „ 10 „	20	16
17 Jan. '11		Cupboard			2 „ 14 „		
					2 „ 17 „		
					2 „ 20 „		
7 Jan. '11—	10	Beehive	44·3 32·2	—	2 „ 10 „	24	15
31 „					3 „ 13 „		
					3 „ 17 „		
					1 „ 21 „		
					1 „ 24 „		
12 Sept. '11	4	Incubator	Dry 75·0	·54	1 „ 3 „	5	4
		75 Dry			2 „ 4 „		
					1 „ 5 „		
12 „	4	Incubator	Dry 84·1	·59	4 „ 3 „	—	—
		85 Dry					



TABLE XLVII. *Adult fleas. X. cheopis. Length of life unfed. Fleas freshly emerged from cocoons, spun in Incubator 75 Wet, placed in filter paper tubes and buried in sand in various situations.*

Date 1911	Number	Place	Temperature max. min.	Humidity	Detail	Longest period of survival, days	Average period of survival, days
7 Oct.	6	Cellar	56·0 54·4	·93	3 dead in 23 days	28	25
					2 „ 27 „		
					1 „ 28 „		
7 „	6	Incubator 75 Wet	74·2	·72	2 „ 7 „	13	10
					1 „ 9 „		
					2 „ 12 „		
					1 „ 13 „		
19 „	10	„ 75 Dry	74·8	·56	5 „ 4 „	6	5
					4 „ 5 „		
					1 „ 6 „		
19 „	10	„ 85 Dry	84·0	·61	1 „ 2 „	5	4
					6 „ 4 „		
					3 „ 5 „		
19 „	10	„ 85 Wet	84·2	·80	1 „ 6 „	13	9
					3 „ 8 „		
					3 „ 9 „		
					2 „ 11 „		
					1 „ 13 „		
19 „	10	Lab. Cupboard	52·5 49·8	·86	2 „ 6 „	17	12
					4 „ 12 „		
					1 „ 14 „		
					3 „ 17 „		
24 „	10	Warm Cupboard Dry	67·0	·55	4 „ 4 „	6	5
					6 „ 6 „		
24 „	10	Beehive	56·2 34·5	—	3 „ 4 „	6	5
					7 „ 6 „		

TABLE XLVIII. *Adult fleas. Ct. canis. Length of life unfed. Fleas, freshly emerged from cocoons, reared in Incubator 75 Wet, from larvae taken from the dog's bed. The fleas were placed in filter paper tubes and buried in sand in various situations.*

Date of emergence and commencement of test	No.	Place	Temperature	Humidity	Detail	Longest period of survival, days	Average period of survival, days
7 Nov. '10	5	Incubator 85 Wet	84.0	.71	5 dead in 6 days	—	—
17 „	9	„	84.0	.70	4 „ 4 „ 5 „ 8 „	8	6
17 „	8	Incubator 75 Wet	74.3	.73	1 „ 5 „ 2 „ 8 „ 4 „ 11 „ 1 „ 13 „	13	9
28 „	13	Warm Cupboard	59.8	.60	3 „ 4 „ 10 „ 6 „	6	5
7 „	5	Cellar (moist corner)	max. 45.0 min. 43.7	over .93	1 „ 12 „ 3 „ 21 „ 1 „ 32 „	32	21
13 Dec. '10	10	Box (out of doors)	No record, there were several sharp frosts	—	4 „ 6 „ 3 „ 10 „ 2 „ 25 „ 1 „ 29 „	29	13
10 Nov. '10	4	Laboratory Cupboard	54.0 42.8	about .84	2 „ 4 „ 1 „ 9 „ 1 „ 11 „	11	6
17 Aug. '11	4	Cellar (moist corner)	Aug. 64.5 63.4 Sept. 60.6 59.5	over .94 „ .93	2 „ 34 „ 2 „ 44 „	44	39
21 Sept. '11	10	Incubator 75 Dry	74.7	.51	4 „ 4 „ 4 „ 5 „ 1 „ 6 „ 1 „ 7 „	7	5
21 „	10	Incubator 75 Wet	74.4	.73	2 „ 5 „ 6 „ 9 „ 2 „ 10 „	10	8
21 „	10	Incubator 85 Dry	84.6	.56	1 „ 2 „ 6 „ 4 „ 3 „ 5 „	5	4
21 „	10	Incubator 85 Wet	84.7	.77	2 „ 5 „ 4 „ 6 „ 4 „ 8 „	8	7

TABLE XLVIII—Continued.

Date of emergence and commencement of test	No.	Place	Temperature	Humidity	Detail	Longest period of survival, days	Average period of survival, days
21 Sept. '11	10	Warm Cupboard	64.2	.66	1 dead in 5 days	10	7
					4 „ 6 „		
					3 „ 7 „		
					1 „ 9 „		
					1 „ 10 „		
21 „	10	Beehive	67.4 41.4	—	3 „ 9 „	20	14
					3 „ 12 „		
					1 „ 15 „		
					2 „ 19 „		
					1 „ 20 „		
22 „	10	Laboratory Cupboard	59.7 51.1	.82	2 „ 8 „	24	14
					1 „ 11 „		
					2 „ 12 „		
					2 „ 13 „		
					1 „ 17 „		
					1 „ 21 „		
					1 „ 24 „		
22 „	30	Cellar	Sept. 60.6 59.5 Oct. 56.3 54.8 Nov. 50.7 48.9	.93 .92 .91	13 „ 31 „ 14 „ 42 „ 1 „ 52 „ 1 „ 54 „ 1 „ 57 „	57	35
					Put into 1½" ento. box and buried in sand.		
21 „	13	Cellar (moist corner)	Sept. 60.6 59.5 Oct. 56.3 54.8	over .93 „ .92	2 „ 15 „ 8 „ 32 „ 3 „ 39 „	39	31

TABLE XLIX. *Adult fleas. C. gallinae. Length of life unfed. Fleas (apparently freshly emerged from cocoon) obtained from empty nest of blue tit, placed in filter paper tubes and kept in sand pots in various situations.*

Date of emergence and commencement of test	No.	Place	Temperature max. min.	Humidity	Detail	Longest period of survival, days	Average period of survival, days
13 Feb. '11	4	Beehive	51.7 32.7	—	2 dead in 21 days	27	26
					1 „ 25 „		
					1 „ 27 „		
„	3	Lab. Cupboard	55.3 45.6	.84	1 „ 14 „	28	20
					1 „ 19 „		
					1 „ 28 „		
„	6	Cellar	46.9 45.3	.92	1 „ 5 „	45	32
					1 „ 35 „		
					1 „ 39 „		
					2 „ 45 „		
					1 escaped		

TABLE L. Adult fleas of various species, length of life unfed. Fleas freshly emerged from cocoons spun in Incubator 75 Wet and kept in the cellar in card jars containing sand for them to burrow in.

Species	Date of emergence and commencement of the test	Number	Place	Temperature	Humidity	Detail	Longest period of survival
<i>P. irritans</i>	20 Dec. '11	12	Cellar			Only one active seen after end of January	84 days
<i>Ct. canis</i>	4 Jan. '12	4	"	max. min. Sept. 60·6 59·5 Oct. 56·3 54·8 Nov. 50·7 48·9 Dec. 48·7 47·4 Jan. 47·2 45·9 Feb. 45·8 44·6 Mar. 49·1 48·1 Apr. 50·3 49·1 May 54·2 53·2 June 56·4 55·6	.93 .92 .91 .92 .92 .92 .93 .93 .93 .93	More than one seen active at end of Feb., all dead by 2 March	58 "
<i>X. cheopis</i>	1 Jan. '12	20	"			Several seen active on 1 Feb., all dead by 7 Feb.	38 "
<i>C. gallinae</i>	1—5 Feb. '12	4	"			3 died between 29 May and 1 June, last died 11 June	127 "
<i>C. fasciatus</i>	5 Sept. '11	20 to 30	"			A number were seen active on 2 Dec. but by the 7th only one feeble individual was to be seen, all were dead by 12 Dec.	about 95 days

# Reports on Plague Investigations in India 623

TABLE LI. *Adult fleas. P. irritans. Length of life unfed after feeding for a period. Fleas freshly emerged were fed for at least one week on human blood and then kept without food in filter paper tubes placed in sand pots in various situations.*

Date of commencement of test	No.	Place	Temperature max.	min.	Humidity	Total length of life, days	Length of life unfed, days
30 Sept. '10	1	Cellar (moist corner)	50·4	49·2	over ·93	121	112
9 Jan. '11	1	Cellar	46·0	44·6	·92	44	37
27 Feb.	1	„	52·3	50·8	·92	130	120
9 Jan.	1	Beehive	44·2	34·8	—	30	23
27 Feb.	1	„	52·2	32·9	—	45	38
9 Jan.	1	Lab. Cupboard	52·4	43·0	·84	44	37
27 Feb.	1	„	54·9	45·3	·85	31	24
9 Jan.	1	Incubator 75 Wet	74·5		·75	23	16
„	1	„ 85 Wet	84·9		·68	21	14
„	1	„ 75 Dry	76·5		·55	17	10
„	1	„ 85 Dry	85·5		·56	12	5

TABLE LII. *Adult fleas. C. fasciatus. Length of life without further food after feeding on rats' blood for varying periods. A special cage was stocked on 28 Dec. '10, with fleas freshly emerged from cocoons kept in Incubator 75 Wet; these fleas were allowed opportunity of feeding upon a rat for 24 hours and upwards, afterwards being kept without food in filter paper tubes placed in sand pots.*

## (a) In Cellar.

Date of commencement of test	No.	Allowed access to rat for	Temperature max.	min.	Humidity	Details	Longest period of survival unfed, days	Average period of survival unfed, days
29 Dec. '10	18	24 hours	46·5	45·3	·93	2 dead in 17 days	33	26
						4 „ 21 „		
						8 „ 27 „		
						1 „ 30 „		
						3 „ 33 „		
31 Dec. '10	15	48 „	46·5	45·3	·93	2 „ 14 „	32	19
						2 „ 19 „		
						3 „ 25 „		
						2 „ 28 „		
						4 „ 31 „		
						2 „ 32 „		
3 Jan. '11	10	72 „	46·5	45·3	·93	2 „ 16 „	30	24
						2 „ 22 „		
						2 „ 25 „		
						2 „ 28 „		
						1 „ 29 „		
						1 „ 30 „		
7 Jan. '11	11	1 week	46·5	45·3	·93	3 „ 18 „	26	21
						5 „ 21 „		
						2 „ 25 „		
						1 „ 26 „		

TABLE LII—*Continued.*(b) *In Beehive.*

Date of commencement of test	No.	Allowed access to rat for	Temperature max. min.	Humidity	Details	Longest period of survival unfed, days	Average period of survival unfed, days
9 Jan. '11	10	9 days	43·5 33·0	—	1 dead in 8 days	19	13
					4 „ 11 „		
					4 „ 15 „		
					1 „ 19 „		

NOTE. Allowance must be made for the fact that the natural death rate might have already eliminated weaklings from the cage before the individuals were taken for the later experiments.

TABLE LIII. *Adult fleas. Ct. canis. Length of life without food. Fleas taken from the dog, put into Incubator 75 Wet at 75° F., Humidity .77 for 24 hours (in order to obtain eggs for experiment) and then kept in filter paper tubes buried in sand pots in various situations.*

Date of commencement of test	No.	Place	Temperature max. min.	Humidity	Detail	Longest period of survival unfed, days	Average period of survival unfed, days
8 Sept. '11	2	Cellar	63 62	.94	all dead in 4 days		
„	2	Lab. Cupboard	73 61	.75	all „ 2 „		
„	2	Beehive	87 54·3	—	1 „ 1 day	3	2
					1 „ 3 days		
„	2	Warm Cupboard	69·0	.66	all „ 2 „	—	—
„	2	Incubator 85 Wet	84·6	.81	1 „ 1 day	3	2
					1 „ 3 days		
„	2	„ 75 Wet	75·0	.79	all „ 1 day	—	—
„	2	„ 75 Dry	75·5	.57	1 „ 1 „	2	—
					1 „ 2 days		
„	2	„ 85 Dry	84·0	.62	all „ 2 „	—	—

TABLE LIV. *Adult fleas. P. irritans. Length of life when fed on man.*

(a) *Freshly emerged fleas, kept in gauze-covered boxes in Laboratory or Bedroom and fed daily.*

Date of emergence	No.	Origin		Length of life approximate*	Remarks
		Situation and food during larval stage	Days in cocoon		
30 Mar. '11	1	{ Lab. blanket } { Cupboard, shakings }	about 211	1 dead about 148 days	Commenced to lay 1 May '11
1 April	1	„ B.S. Rag	„ 160	1 „ 160 „	
10 „	1	„ Fleafaeces	„ 228	1 „ 161 „	
18 „	1	„ B.S. Rag	„ 200	1 „ 153 „	
21 „	1	„ „	„ 203	1 „ 250 „	
22 „	1	„ „	„ 215	1 „ 251 „	
27 „	1	„ „	„ 220	1 „ 316 „	

\* Those which were first to emerge are considered to be the first to die.

TABLE LIV—Continued.

(b) *Freshly emerged fleas, kept in gauze-covered boxes in Laboratory Cupboard and fed twice weekly.*

Date of emergence	No.	Temperature max. min.	Humidity	Length of life approximate
22 Dec. '10	2	Jan. 53·5 44·0	·84	1 dead in 75 days *
24 „	1	Feb. 52·8 43·6	·83	1 „ 204 „
25 „	1	Mar. 55·2 45·5	·85	2 „ 226 „
27 „	2	April 58·2 48·2	·78	1 „ 231 „
		May 61·8 54·3	·78	1 „ 239 „
		June 66·4 58·3	·79	
		July 71·5 62·7	·75	
		Aug. 72·0 64·1	·74	

(c) *Freshly emerged or captured fleas, kept in gauze-covered boxes in Laboratory or Bedroom and fed daily.*

Date of emergence or capture	No.	Origin	Length of life	Sex	Longest period of survival, days	Average period of survival, days
July '10	2	Captured	1 dead in 100 days	♀	162	130
			1 „ 162 „	♀		
Aug. '10	4	Bred in Lab. Cupboard freshly emerged	1 „ 131 „	♀	142	135
			1 „ 142 „	♀		
			1 „ 135 „	♂		
			1 „ 135 „	♂		
3 Oct. '10	1	Captured	1 „ 58 „	♀	—	—
21 Feb. '11	18	Bred in Lab. Cupboard, freshly emerged (larvae taken from dog's bed Oct. '10)	1 „ 148 „	—	513	348
			1 „ 160 „			
			2 „ 177 „			
			1 „ 190 „			
			1 „ 360 „			
			2 „ 377 „			
			2 „ 388 „			
			1 „ 397 „			
			1 „ 424 „			
			2 „ 434 „			
			1 „ 437 „			
			2 „ 443 „			
			1 „ 513 „			

\* This flea was found dead gummed to glass of the box by blood voided by the fleas; it is possible but not definite that this was the cause or contributory cause of death.

TABLE LIV (c).—*Continued.*

Date of emergence or capture	No.	Origin	Length of life		Sex	Longest period of survival, days	Average period of survival, days
3 June '11	10	Bred in Cellar, freshly emerged (larvae taken from dog's bed Oct. '10)	1	dead in 49 days	—	269	126
			1	„ 72 „			
			2	„ 80 „			
			2	„ 84 „			
			1	„ 100 „			
			1	„ 192 „			
			1	„ 258 „			
			1	„ 269 „			
20 June '11	17	Bred in Incubator 75 Wet freshly emerged	1	„ 58 „	—	252	112
			6	„ 67 „			
			4	„ 87 „			
			2	„ 99 „			
			1	„ 203 „			
			1	„ 206 „			
			1	„ 241 „			
			1	„ 252 „			
Fed twice a week.							
9 Mar. '11	7	Freshly emerged	4	„ 92 „	—	166	121
			1	„ 153 „			
			1	„ 158 „			
			1	„ 166 „			

(d) *Kept in the Cellar and fed daily until 2nd October and then once a week.*

23 Aug. '11	11	Freshly emerged	1	„ 103 „	—	511	281
			1	„ 192 „			
			2	„ 200 „			
			1	„ 206 „			
			1	„ 278 „			
			2	„ 345 „			
			1	„ 414 „			
			1	„ 505 „			
			1	„ 511 „			



TABLE LV. *Adult fleas. Length of life of various species when fed on rats. Fleas freshly emerged from cocoons in Incubator 75 Wet and kept in gauze-covered boxes in various situations.*

Species	Method of feeding	Date of emergence	Number	Place	Temperature max. min.	Humidity	Length of life 2 dead in 24 days	Aver. 28 days
<i>C. fasciatus</i>	Box method, 15 minutes daily	7 Oct. '10	5	Rat's cage	—	—	3 " 31 "	28 "
"	"	7 Feb. '11	10	Lab. Cupboard	56·7 47·7	·81	1 " 8 " 1 " 9 " 5 " 12 " 1 " 14 " 1 " 90 " 1 " 106 "	28 "
"	Box method, 15 minutes every other day	12 Oct. '10	9	Incubator 85 Wet	83·9	·71	1 " 4 " 1 " 10 " 4 " 12 " 2 " 18 " 1 " 20 "	13 "
<i>P. irritans</i>	Box method, 15 minutes daily	4 Feb. '11	6	Lab. Cupboard	52·0 43·7	·84	1 " 3 " 1 " 6 " 1 " 8 " 1 " 9 " 1 " 11 " 1 " 14 "	8 "
"	In cage with rat	30 Nov. '10	6	—	—	—	The last living specimen was seen 40 days from start	
<i>Ct. canis</i>	Box method, 15 minutes daily	17 Nov. '10	10	Lab. Cupboard	58·4 45·4	·85	1 dead in 24 days 1 " 30 " 3 " 34 " 1 " 46 " 1 " 48 " 2 " 54 " 1 " 58 "	41 days
"	In cage with rat	27 Nov. '10	60	—	—	—	No record of individual deaths; they died off gradually, all were dead by 25 Dec. '10	

TABLE LVI. *Adult fleas, C. fasciatus and X. cheopis. Length of life when fed on man. Fleas freshly emerged from cocoons in Incubator 75 Wet, fed daily and kept in Laboratory Cupboard during the day and in Bedroom at night.*

Species	Date of emergence	Number	Length of life	Longest period of survival, days	Average period of survival, days
<i>C. fasciatus</i>	3 Oct. '10	6	1 dead in 17 days	79	39
			2 ,, 19 ,,		
			1 ,, 22 ,,		
			2 ,, 79 ,,		
„	18 Oct. '10	4	1 ,, 4 ,,	76	35
			1 ,, 19 ,,		
			1 ,, 41 ,,		
			1 ,, 76 ,,		
„	15 July '11	12	*10 ,, 13 ,,	62	18
			1 ,, 26 ,,		
			1 ,, 62 ,,		
<i>X. cheopis.</i>	15 July '11	19	3 ,, 18 ,,	100	38
			†12 ,, 33 ,,		
			1 ,, 55 ,,		
			1 ,, 65 ,,		
			1 ,, 67 ,,		
			1 ,, 100 ,,		
			approximate		
„	8 June '11	2	1 ,, 55 ,,	87	73
	13 ,,	4	2 ,, 66 ,,		
	11 July '11	9	4 ,, 69 ,,		
			1 ,, 70 ,,		
			3 ,, 71 ,,		
			4 ,, 87 ,,		

\* 10 all died at one time, cause of death unexplained, possibly due to entanglement of legs in piece of cloth put into box to induce females to lay.

† All died on the same date. When removed from the box they were found to have their legs entangled in the cloth; whether this had any relation to their death or if it occurred subsequently to death is not known.

TABLE LVII. Adult fleas. Various species. Length of life when fed daily on man.

Species	Date of emergence or capture	Feeding	Origin	No.	Length of life	Longest period of survival, days	Average period of survival, days	Remarks
<i>Ct. canis</i>	30 Oct. '10	Box method, 15 minutes daily	Captured on Dog	3	1 dead in 38 days	62	51	—
"	7 Nov. '10	"	Bred (freshly emerged)	6	1 " 52 "	245	72	—
"	17 May '11	"	"	8	2 " 15 "	345	145	—
					1 " 31 "			
					1 " 45 "			
					1 " 84 "			
					1 " 245 "			
					2 " 66 "			
					2 " 84 "			
					1 " 89 "			
					1 " 156 "			
					1 " 275 "			
					1 " 345 "			
<i>Ct. felis</i>	29 Dec. '10	"	"	5	1 " 139 "	185	156	—
					1 " 146 "			
					1 " 152 "			
					1 " 157 "			
					1 " 185 "			
<i>C. gallinae</i>	7 Nov. '10	"	Captured on Dog	1	1 " 224 "	—	—	This specimen was a female, she laid a few infertile eggs during the spring of 1911
"	12 Feb. '10		Captured in nest of Blue Tit (apparently freshly emerged)	7	1 " 6 "	41	18	These fleas were observed to copulate before feeding commenced. *One female laid fertile eggs after some weeks captivity. 11 fleas were reared from these eggs
					1 " 8 "			
					2 " 15 "			
					1 " 22 "			
					1 " 25 "			
					*1 " 41 "			
<i>L. musculi</i>	31 May '12	15 to 20 minutes daily	Bred in Incubator 75 Wet, larvae taken from cages (freshly emerged)	11	5 " 4 "	10	6	One noted very full of blood
					2 " 5 "			
					3 " 8 "			
					1 " 10 "			
"	19 April '12	"	"	6	2 " 3 "	5	4	"
					3 " 4 "			
					1 " 5 "			
<i>Spilopsyllus cuniculi</i>	12 April '12	"	Reared from a rabbits' nest	19	9 " 13 "	69	27	Kept under living room conditions when not feeding
					3 " 24 "			
					4 " 38 "			
					1 " 52 "			
					1 " 56 "			
					1 " 69 "			

TABLE LVIII. *Adult fleas. Ct. canis and C. fasciatus. Length of life without food when kept in various situations and supplied with a small piece of blotting paper moistened with distilled water each night.*

Species	Date of emergence or capture	Origin	No.	Place kept during test	Temperature max. min.	Humidity	Detail	Longest period of survival, days	Average period of survival, days
<i>C. fasciatus</i>	18 Nov. '10	Bred, freshly emerged	4	Lab. Cupboard	55.0 45.6	.85	1 dead in 9 days	48	27
							1 " 12 "		
							1 " 39 "		
							1 " 48 "		
	4 "	Captured	4	"	54.0 42.8	.84	1 " 21 "	26	23
							2 " 24 "		
							1 " 26 "		
<i>Ct. canis</i>	17 "	Bred, freshly emerged	10	Incubator 85 Wet	Dry 83.9	.71	1 " 4 "	10	8
							5 " 8 "		
	17 "	"	10	" 75 Wet	74.3	.73	4 " 10 "	15	10
							1 " 4 "		
							3 " 10 "		
							1 " 12 "		
							1 " 15 "		
	17 "	"	9	Warm Cupboard	59.5	.57	(Remainder escaped)	21	17
							1 " 11 "		
							2 " 14 "		
							3 " 17 "		
							1 " 18 "		
							1 " 20 "		
							1 " 21 "		
	17 "	"	8	Lab. Cupboard	55.3 46.2	.85	1 " 11 "	34	20
							2 " 15 "		
							1 " 18 "		
							2 " 22 "		
							1 " 27 "		
							1 " 34 "		
	17 Feb. '11	"	13	Incubator 75 Dry	75.6	.51	1 " 3 "	10	6
							6 " 4 "		
							2 " 5 "		
							1 " 7 "		
							1 " 9 "		
							2 " 10 "		
	21 "	"	10	" 85 Dry	83.9	.59	4 " 3 "	10	5
							4 " 6 "		
							1 " 9 "		
							1 " 10 "		

TABLE LIX. *Adult fleas. Ct. canis and C. fasciatus. Length of life of freshly emerged specimens, when supplied with various artificial foods. Liquid food supplied on pad of blotting paper or greasy sheeps' wool put into their boxes and remoistened each night.*

Species	Nature of liquid supplied	Date of emergence	No.	Place kept during test	Temperature max. min.	Humidity	Length of life dead in 4 days	Longest period of survival, days	Average period of survival, days
<i>Ct. canis</i>	(A) Broth on blotting paper	17 Aug. '11	10	Lab. Cupboard	70·5 61·8	·76	5	22	8
							1 " "	5	
							1 " "	7	
							1 " "	12	
							1 " "	18	
							1 " "	22	
	(B) Water on blotting paper	15 Aug.	14	"	70·3 61·8	·75	1 " "	6	13
							2 " "	7	
							3 " "	9	
							1 " "	12	
							1 " "	15	
							3 " "	16	
							1 " "	18	
							1 " "	20	
							1 " "	26	
<i>C. fasciatus</i>	(A) Broth with sugar on blotting paper	26 May	10	"	68·8 61·4	·76	1 " "	4	6
							3 " "	5	
							1 " "	6	
							5 " "	8	
	(B) Water on blotting paper	26 May	10	"	66·6 58·9	·79	1 " "	12	22
							1 " "	14	
							1 " "	16	
							1 " "	17	
							1 " "	22	
							2 " "	23	
							1 " "	29	
							1 " "	42	
							1 escaped		

TABLE LIX.—Continued.

Species	Nature of liquid supplied	Date of emergence	No.	Place kept during test	Temperature max. min.	Humidity	Length of life	Longest survival, days	Average period of survival, days
<i>C. fasciatus</i>	(A) Broth with sugar on blotting paper	29 June	12	Lab. Cupboard	68.1 60.1	.80	6 dead in 8 days 5 " 10 " 1 " 12 "	12	9
"	(A) Broth with sugar on pad of wool	29 May	8	Incubator 75 Wet	74.2	.91	1 " 1 day 7 " 2 days	2	2
"	(B) Water on pad of wool	30 May	10	"	75.9	.80	1 " 1 day 2 " 3 days 1 " 4 " 5 " 6 " 1 escaped	6	4
"	(A) Broth with sugar on pad of wool	1 June	11	Cellar	59.2 57.7	.93	1 dead in 2 2 " 4 " 3 " 5 " 2 " 7 " 3 " 13 " 2 " 6 " 6 " 12 " 2 " 13 "	13	7
"	(B) Water on pad of wool	2 June	10	"	58.8 57.2	.93	2 " 6 " 2 " 12 " 2 " 13 "	13	11
"	(A) Broth on blotting paper	10 July	9	Lab. Cupboard	70.6 60.8	.74	1 " 3 " 7 " 5 " 1 " 6 "	6	5
"	(B) Water on blotting paper	10 July	7	"	72.9 63.7	.73	1 " 11 " 2 " 16 " 2 " 21 " 2 escaped	21	17
"	(B) " " "	15 Aug.	35	Incubator 75 Dry	74.7	.59	2 dead in 4 3 " 6 " 8 " 7 " 7 " 8 " 6 " 9 " 2 " 10 " 5 " 11 " 1 " 13 " 1 " 15 "	15	8

v. *Possibility of continued breeding in the absence of any host.*

Some experiments were made in order to see whether fleas could establish themselves in the absence of a host, by throwing successive broods, the necessary nutriment for reproduction by the adults being obtained, as in case of so many insects, by the surplus passed on from the larval period. Many of the experiments with adults, although performed for other purposes, afford evidence bearing on this subject, but the procedure was arranged in these special tests with a view to making the attendant circumstances as favourable as possible. The evidence obtained is entirely negative in character.

*P. irritans*. A large glass jar was stocked with a quantity of carpet sweepings and greasy unwashed sheep's wool. Some 270 newly hatched larvae were added and the jar was placed in the cellar on the 22nd May, 1911. Periodic examinations were made from 23rd September, 1911, onwards to September, 1912. A few adult fleas were seen during September and October, 1911, but none afterwards and no trace of larvae could be found.

*C. fasciatus*. A similar glass jar, stocked in the same way, was placed in the cellar on the 30th May, 1911, and 110 newly hatched larvae of *C. fasciatus* were added. A number of adult fleas (36 in all) were found from time to time up to the 10th May, 1912. Subsequent examinations up to September, 1912, were barren and no trace of any larvae could be found.

*C. gallinae*. Material taken from a nesting box used by a blue tit was received in June, 1911. It was kept in a glass jar in the beehive. Neither larvae nor fleas could be found when it was taken from the box. An examination in September, 1911, showed that some *C. gallinae* had emerged; cocoons were also discovered and opened and found to contain living fleas. Fleas continued to emerge up to the 24th April, 1912, but no larvae could be detected at any time up to September, 1912. Another blue tit's nest, received on the 9th October, 1911, and kept in the beehive, gave a similar record—the last living flea being found on the 7th March, 1912; but no larvae could be discovered.

A further experiment was made with *P. irritans* starting with freshly emerged adults. A large glass jar was prepared with a quantity of carpet sweepings and greasy unwashed sheep's wool and placed in the cellar on 22nd May, 1911. 20 freshly emerged *P. irritans* were fed for one week, then kept in a box in the cellar for a further week, to see if any ova would be laid immediately after the feeding. No ova were laid and the fleas were then put into the jar mentioned above.

Examinations were made periodically from September, 1911, to May, 1912, but no signs of either larvae or fleas could be found.

The cellar was chosen for the above experiments as, in consequence of the long survival period of living fleas kept there, it was thought to afford the most favourable situation for continued breeding. In order to test the possibility of egg laying under conditions exactly similar to those used to obtain eggs from fed captives, the following experiment was performed with unfed adult fleas.

A number of freshly emerged males and females of *P. irritans*, *X. cheopis* and *C. fasciatus* were placed in boxes having the cloth ring (as described in the general chapter on Methods, p. 464) and put away as follows:

Warm Cupboard. The boxes containing the fleas were kept in a moist chamber (temperature about 64° F. and the air kept as near saturation as possible).

Date	No.	Species	Remarks
15 Aug. '12	30	<i>P. irritans</i>	by the 31 Aug. all had died, no eggs were laid
"	30	<i>X. cheopis</i>	" " " "
"	30	<i>C. fasciatus</i>	" " " "

Incubator 75 Wet. Boxes containing the fleas were buried in a mass of carpet sweepings (fluff and dust) (temperature about 74° F., humidity about .81).

15 Aug. '12	15	<i>P. irritans</i>	by the 30 Aug. all had died, no eggs were laid
"	30	<i>X. cheopis</i>	" " " "
"	30	<i>C. fasciatus</i>	" " " "

Incubator 75 Wet. Boxes containing the fleas were buried in sand (temperature about 75° F., humidity .75 to .80).

18 Sept. '12	30	<i>C. fasciatus</i>	by the 5 Oct. '12 all were dead, no eggs were laid
"	30	<i>X. cheopis</i>	" 28 Sept. '12 all had died, " "

In further support of this negative result it may be noted that when flea breeding cages are kept for more than two or three months without a host for the fleas to feed on, adults will still be found present but no larvae. Cages used for breeding *C. fasciatus* were found to contain imagines 10 months after the removal of the rat but no active larvae were ever discovered.

This question is discussed at greater length in the general chapter dealing with the bionomics of fleas, Section IV, pp. 477—480.

vi. *Fertility of males and survival of spermatozoa in the spermathecae of the female.* *P. irritans*.

My interest in Röscl von Rosenhoff's (1749) statement that fleas only copulate three times—the males not being able to fertilize after the third occasion—led me to make the following observations on captive specimens of *P. irritans*.



A single male of *P. irritans* was kept with four or five females for over a month, and though eggs were removed every three or four days there was no occasion on which all the eggs were infertile.

Two females were kept in a box with a male; the male was then taken away on the 24th July, 1910, and the females transferred to a fresh box. The change to a fresh box was repeated at intervals.

Eggs laid 28th July	to	1st August—none hatched
„ 2nd August „ 5th	„	„ „ „
„ 6th „ „ 11th	„	„ „ „
„ 12th „ „ 14th	„	„ „ „

Later a male was then added to the above mentioned two females with the following results:—

26th September 1910: 6 eggs laid, but did not hatch.

1st October 1910: 12 eggs laid from which 11 larvae hatched.

A further trial was made as follows:—

6th October 1910: The same two females and a male—10 eggs laid, at least two larvae hatched.

15th „	as above—12 eggs laid, 7 larvae hatched.
22nd „	„ 7 „ 1 larva „
25th „	„ 5 „ none „
1st November, 1910: „	6 „ none „
8th „	„ 4 „ 2 larvae „

Twelve eggs were laid by a captured female some five days to a week after capture—one egg hatched.

A week or 10 days later this female laid 6 more eggs, having had no intercourse with a male—none of the eggs hatched.

Seven eggs which were laid by a virgin female kept and fed in a separate box did not hatch.

The suggestion to be gleaned from these observations was that, as a single male fertilized the eggs of at least one of a number of females for more than a month, Rösel von Rosenhoff's statement, alluded to above, must be incorrect, and further that females could not continue to lay fertile eggs after separation from the male for a few days.

The following experiment, however, made in the autumn of 1911, shows that the last conclusion was incorrect, and that females may retain the power of laying fertile eggs for a considerable period after copulation.

A number of freshly emerged females and males were put into a box and fed for 12 days, when all the males were removed and the females' oviposition recorded. The latter were kept at 75° F. when not feeding.

*Bionomics of Fleas*

Date	Number of ova laid	Number hatched
19th October 1911	18	Nil
21st    ,,	10	2
25th    ,,	42	19
28th    ,,	38	7
2nd November 1911	44	15
6th    ,,	40	1
10th    ,,	8	Nil
14th    ,,	10	1
18th    ,,	16	Nil
24th    ,,	11	Nil
28th    ,,	8	Nil
2nd December 1911	9	3
9th    ,,	7	1
14th    ,,	11	Nil
19th    ,,	10	Nil

The following two series of experiments were then put in hand with a view to settling the questions raised by the earlier trials, the object being to test the fertilizing power of a male and to determine the length of time during which the female retains the power of laying fertile eggs without access to a second male.

The method employed was the following: a single male was selected out of a number of freshly emerged fleas and allowed to remain at least four days in succession with each of a number of females freshly emerged from cocoons that had been kept in separate tubes to avoid any possibility of random pairing.

The ova were removed from the boxes every three or four days. All the specimens when not feeding were kept in Incubator 75 Wet.

*Fertility experiment with P. irritans. SERIES I.*

The male used emerged from cocoon on 3 Feb. 1912.

Female No.	Date of emergence from cocoon	Male added	Male removed	Started to lay	No. of eggs laid	Number hatched	Remarks
1	3 Feb. '12	5 Feb. '12	12 Feb. '12	4 Mar. '12	2 2	0 0	
2	3    ,,	12    ,,	16    ,,	26 Feb. '12	1 1 1	0 0 0	
3	3    ,,	16    ,,	20    ,,	24    ,,	2 2	0 0	
4	3    ,,	20    ,,	24    ,,	—	—	—	did not lay
5	3    ,,	24    ,,	28    ,,	8 Mar. '12	2 7	0 0	
6	3    ,,	28    ,,	3 Mar. '12	5    ,,	6 3 1	0 0 0	
7	16    ,,	3 Mar. '12	7    ,,	8    ,,	4 5	0 0	
8	19    ,,	7    ,,	11    ,,	16    ,,	3	0	
9	20    ,,	11    ,,	15    ,,	16    ,,	6	3	
10	29    ,,	15    ,,	19    ,,	—	—	—	did not lay

SERIES No. I. In the experiments of the first series (see Table above), none of the eggs laid by the first eight females hatched. It was therefore decided to abandon the experiment, on the ground that

the male was in some way defective. This decision had already been acted on and the females added to the general stock before the ova laid by female No. 9 had hatched.

SERIES NO. II. The second series was successfully carried through. The results are of considerable interest, and show the large amount of variation that exists both in regard to the egg laying capacity of the females of *P. irritans* and the fertilizing powers of the males. While the conditions of the experiment were not exactly natural they were not unfavourable, and are likely to differ from what obtains under free conditions only as regards the restriction of the opportunities for copulation. As a measure of variation between one specimen and another the results of the experiments seem perfectly valid.

The result to be gleaned from Series II (*a*), April to June 1912 (see Table on pp. 639, 640), is that a single male is able to fertilize as many as 13 females, but that the supply of sperms passed to the female becomes exhausted within a period of from one to two months. In order to make sure that the failure of the eggs to hatch was due to the absence of a male and not to some defect in the females, a second male was placed with females Nos. 1, 2, 3, 9, 13 and 14 after they had each laid at least six batches of infertile eggs, that is after a period of about 25 to 30 days had elapsed since any fertile ova had been laid.

The sequel Series II (*b*) to Series II (*a*) lasted from June to December 1912, and the results entirely confirm those previously obtained. The course of the experiment was interrupted by a change in the food from the middle of July to the end of August 1912, when the insects were fed by my assistant during my absence from home. It will be noted that egg laying fell off at about this period, possibly owing to the change of diet, but more probably either because they did not get such full meals, or because there was a considerable fall in mean temperature during the latter half of July and throughout August, 1912. Upon my return I decided to keep the insects in incubator 75 Wet in order to finish the fertility observations, and I also instituted a separate experiment to test the effect of differential feeding.

Towards the close of the experiment the daily periods allowed for feeding were considerably increased and the eggs were allowed to accumulate for periods of 5, 6 or even 7 days before removal; it is to these causes that the apparent spurt in egg laying (see for example, females Nos. 13 and 14 in Series II (*b*)) is to be, at any rate in part, attributed, but the number of collapsed and small sized eggs suggested also some failure of control in oviposition. This may have been due

either to old age or perhaps to the unnatural condition of long continued warmth and high feeding in place of the normal state of rest or partial hibernation that occurs with this species during the winter.

The introduction of a third male in the case of females Nos. 1, 9, 13 and 14 after the lapse of the period of fertility due to the second copulation, resulted again in the production of fertile eggs by females Nos. 1, 13 and 14. It seems reasonable to suppose that the failure in the case of No. 9 was due to some defect in coitus rather than to failing powers on the part of the female to produce ova capable of fertilization. That the fault lay with the female rather than with the male seems definite as the later introduction of a fourth male did not alter the state of affairs.

It will be seen that female No. 13 was by far the most productive of those tested; she laid 448 eggs, of which 115 were fertile, in a total period of 196 days. This specimen is also to be credited with the ability to retain the power of fertilizing her eggs for the longest period, 65 days.

Fertility experiment with *P. irritans*. SERIES II (a).

The first male used emerged 1 April 1912.

Female No.	1	2	3	4*	5*	6*	7*	8*	9	10†	11†	12	13	14	15
Date of emergence from cocoon 1912	1 April	3 "	9 "	16 "	16 "	16 "	22 "	25 "	25 "	2 May	14 "	19 "	28 "	1 June	6 "
Male added 1912	5 April	9 "	13 "	17 "	21 "	25 "	29 "	3 May	7 "	11 "	15 "	19 "	24 "	1 June	6 "
Male removed 1912	9 April	13 "	17 "	21 "	25 "	29 "	3 May	7 "	11 "	15 "	19 "	28 "	1 June	6 "	12 "
Started 1912 to lay	12 April	13 "	18 "	23 "	30 "	28 "	1 May	5 "	11 "	16 "	22 "	8 June	6 "	10 "	12 "
Number of eggs laid, 4 day totals	6 9 9 11 8 6 4 3 3 5 2 8 5 3 5	6 6 4 6 8 7 8 8 6 2 2 2 0 0 4 4	1 4 7 3 5 9 6 3 3 4 0 4 6 3 3	5 7 10 6 10 6 5	3 3 3 2 3	7 11 10 11 14	4 7 7 7 3	6 7 6 6	8 8 11 7 5 6 4 7 5 4 3 0 3 0 6 3 6 0 0 0	3 3 3 4 4 4 6	4 7 7 7 3	4 4 8 5 6 4 5 6	11 9 6 7 14 8 10 5 5 10 5 3 6 1 0 2 0 0	9 5 4 11 7 0 5 1 6 6 0 4 0 0 3 0 0	2 0 3 7 0 5
Number of eggs which hatched, 4 day totals	4 6 4 5 5 2 0 0 0 0	3 5 2 3 5 4 4 3 3 2 0 0 0 0 0 0	0 3 2 1 0 1 1 2 0 0 0 0 0 0 0 0	3 6 5 3 4 4 1	1 1 2 0 2	4 3 7 5 4	1 6 4 4 2	3 3 3 3	4 4 10 5 4 2 3 0 3 3 1 0 0 0 0 0 0 0 0 0	3 1 1 3 3 2 3	0 2 5 3 2	0 0 0 0 0 0 0	5 5 4 6 6 4 5 3 1 0 0 0 0 0 0 0 0	0 3 2 7 3 0 2 0 1 0 0 0 0 0 0 0 0	0 0 0 0 0 0
Effective period of fertilization	24 days	40 "	32 "	—	—	—	—	—	44 days	—	—	—	36 days	36 "	—
Total number of eggs	87 in 62 days	73 "	61 "	49 "	14 "	53 "	28 "	25 "	86 "	27 "	28 "	42 "	93 "	61 "	17 "
Number of eggs fertilized	26	34	10	26	6	23	17	12	39	16	12	none	39	18	none
Percentage of fertile ova laid, calculated for period of fertility only	53 %	55	26	53	42	42	60	48	57	58	42	—	51	37	—
Remarks	—	—	Copulation observed	—	—	Copulation observed	—	—	Copulation observed	—	Copulation observed	Copulation observed	Flea removed from experiment	—	Male died 12 June '12 while in box with No. 15. Flea removed from experiment

\* Numbers 4 to 8 removed from the experiment on 20 May '12 in order to make room for fresh females.

NOTE. Owing to the time involved and the difficulties in feeding when so large a number of separate boxes were required it was found necessary to limit the experiment. Nos. 4, 5, 6, 8, 10, and 11 were therefore removed on the 20th May (as events happened rather unnecessarily, as the male brought the series to a close by dying on the 12th June).

+ Numbers 10 and 11 also removed.

*Fertility experiment with P. irritans. SERIES II (b).*

Second male, emerged 13 June '12, died after having been placed with females Nos. 1, 2 and 3. Another male, emerged 1 Aug. '12, was used for Nos. 9, 13 and 14.

No.	Female	Male added 1912	Male removed 1912	Number of eggs laid, 4 day totals	Number of eggs hatched, 4 day totals	Number of eggs which hatched, 4 day totals	Effective period of fertilization	Total number of eggs	Number of eggs fertilized	Percentage of fertile ova laid, calculated for period of fertility only	Remarks
No. 1	1	13 June	22 June	7 10 12 9 10 7 7 15 5 3 0 0	1 3 5 5 8 1 7 11 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0	40 days	99 in 88 days	42	49%	—
"	2	22 "	26 "	0 1 3	0 0 0	0 0 0	Nil	4 " 25 "	Nil	—	Died 17 July '12
"	3	26 "	1 July	11 9 10 8 10 3 5 4 1	4 4 4 0 0 0 0 0	0 0 0	16 days	61 " 57 "	12	40	" 22 Aug. '12
"	9	1 Aug.	1 Sept.	2 0 3 0 0 3 * 9 0 6 3 3 2 2	0 0 0 0 0 0 4 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	doubtful	34 " 56 "	4	doubtful	—
"	13	1 Sept.	11 "	* 8 11 12 10 8 10 7 6 4 5 0 8	4 9 10 10 6 9 5 5 2 1 0 2	0 0 1 2 0 0 0 0 0 0	about 65 days	279 " 90 "	75	58	—
"	14	11 "	16 "	0 7 9 8 0 6 18 28 26 56 32	0 0 1 2 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	—	—	—	—	—
Third Male, freshly emerged.											
"	1	21 "	2 Oct.	7 10 5 10 7 10 4 4 3	6 6 3 8 4 7 2 0 0	0 0 0 0 0 0 0 0 0 0	" 30 "	60 " 36 "	36	60	Died 28 Oct. '12
"	9	10 Oct.	20 "	2 2 0 1 5 0 4 5 0 5 11 17 13	0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	Nil	82 " 70 "	Nil	—	" 2 Feb. '13. A fourth male tried but no eggs were laid
"	13	29 Nov.	18 Dec.	27 10 27 12	0 0 1 0	0 0 1 0	doubtful	76 " 20 "	1	1	Died 2 Feb. '13
"	14	29 "	18 "	19 30 7 14	1 5 0 0	1 5 0 0	8 or 10 days	70 " 16 "	6	8	" 27 Dec. '12

\* This mark is placed against a batch of eggs to signify that from this time onwards the females were kept in an incubator at 70°F. It was necessary to pursue this course as the cold weather had caused a cessation of egg laying.

vii. *Influence of food supply upon the number and fertility of eggs.*  
*P. irritans.*

The following experiment was specially arranged to investigate the influence of the amount of food provided for the adult upon the number and fertility of the eggs laid. The results do not exhibit any clear connection between the opportunities afforded for feeding and the percentage of eggs hatching, but, on the other hand, show very definitely the extent to which the total number of eggs laid is dependent on food supply.

*Experiment with P. irritans. The influence of food supply upon  
 (a) the number of eggs laid and (b) their fertility.*

*Method.* A number of freshly emerged fleas were fed for a few days. 5 males and 5 females were selected and then put into each of two boxes. The specimens in Box No. 1 were fed once a day, those in Box No. 2 twice a day.

Box No. 1						Box No. 2					
No. of eggs laid, 3/4 day totals			No. of eggs hatch- ing, 3/4 day totals			No. of eggs laid, 3/4 day totals			No. of eggs hatch- ing, 3/4 day totals		
23	36	49	16	44	12	25	30	11	38		
Total 168			Total 116			Total 277			Total 176		
			69%						63%		

A female in Box No. 2 died and one was taken out of Box No. 1 for the sake of uniformity.

The feeding was reversed after the 5th batch of eggs had been removed. The individuals in Box No. 1 were fed twice a day and those in Box No. 2 once per day.

50	62	44	76	57	34	45	34	54	42	50	44	30	47	24	31	27	26	41	21
Total 289					Total 209					Total 195					Total 146				
					71%										75%				

SECTION VI. INFLUENCE OF LOW TEMPERATURE UPON  
 THE VARIOUS STAGES.

*Ice Chest Experiments* (Table LX). *X. cheopis* bred too slowly in the cages during the cool months of the year to afford a plentiful supply of the insect in all its stages. In consequence experiments with this species were practically restricted to the summer and autumn months and, in order to investigate in detail the influence of low temperature upon the various stages in its life history, recourse was had to a small ice chest kept in the cellar. It was thought well to try *P. irritans* and *C. fasciatus* at the same time for purposes of comparison. The humidity was high—bordering on saturation—and the temperature fairly constant at about 40° F., save on a few occasions when the local supply of ice failed. In this case the extreme range was

between 35° F. and 45° F., mounting for a few hours a day to 50° F. and on one occasion to 54° F.

The results afford further evidence of greater susceptibility to low temperatures on the part of *X. cheopis* than in the case of *P. irritans* and *C. fasciatus* that was apparent in the breeding experiments and also carried out during the autumn of 1911 (Table XXV, p. 532). In all stages, save the imaginal, low temperatures below 45° F. were fatal to *X. cheopis*. As a means of destroying brood, cold air might be used as successfully as any of the usual insecticides against this species, and no further explanation is required for its failure to establish itself in cool or cold climates.

These experiments also bring out clearly the difference between *P. irritans* and *C. fasciatus* in their ability to breed under cool conditions. The record of all three species is the more interesting if the results of this experiment are compared with those obtained previously under the moderate temperatures (47—55° F.) afforded in the cellar.

*Egg stage* (Table LX (a)). While *C. fasciatus* can hatch at an average temperature of 41° F., *P. irritans* cannot, but a rise of 8 or 9° F. allows a percentage to hatch (see Table VI). To induce ova of *X. cheopis* to hatch a temperature of over 60° F. is apparently necessary (see Table VII, p. 497).

*Larval stage* (Table LX (b)). Here again it will be noted that, while *X. cheopis* and *P. irritans* cannot survive below 40° F., *C. fasciatus* is not only able to endure but apparently finds this temperature quite suited to its needs. The only factor that prevented all the larvae being reared to cocoon stage was the length of time taken by lagging larvae.

*Cocoon stage*. At 40—43° F. the results were similar to those obtained with the larvae. The greater endurance of *P. irritans* than *X. cheopis* appears from the fact that five of the former reached the pupal or imaginal stage before death, but only one of the latter survived the larval period.

*Adult stage*. The disparity here is not nearly so marked, *X. cheopis* being nearly or quite as hardy as *P. irritans*, while all three species are more nearly alike in this stage than in any previous one.



TABLE LX. *Ice chest experiment. Relative endurance at low temperature of X. cheopis, P. irritans and C. fasciatus at various stages in their life history.*

General record of Temperature and Humidity during the experiment.

Month	Temperature			Humidity
	Highest	Lowest	Average	
April '12	49	35	(Average of 31 readings)	.92
May	54	35	( " 53 " )	.95
June	50	35	( " 50 " )	.99
July	60	37	( " 43 " )	.95

(a) Eggs, laid in Incubator 75 Wet.

Date	Species	Number	Temperature		Humidity	Number hatched	Mortality 100%
			Extremes	Aver.			
7 June '12	<i>X. cheopis</i>	10	50°	35°	40.9	Nil	100
"	<i>P. irritans</i>	10	50°	35°	40.9	Nil	100
"	<i>C. fasciatus</i>	10	50°	35°	40.9	5	50

(b) Larvae, newly hatched in Incubator 75 Wet, unfed, placed in small glass tubes containing a little sand and plugged with cotton wool.

Date	Species	Number	Temperature		Humidity	Length of life, days		Average length of life
			Extremes	Aver.		Minimum	Maximum	
29 April '12	<i>X. cheopis</i>	6	40°	36°	38.2	.97	2	3
"	<i>P. irritans</i>	9	49°	35°	37.0	.95	2	46
"	<i>C. fasciatus</i>	8	54°	35°	39.0	.96	9	52

Active (feeding) larvae of various sizes taken from the cages (or in the case of *P. irritans* from stock pot in Incubator 75 Wet) and placed in card jars with sand and blood-soaked rag.

Date	Species	Number	Temperature		Humidity	Number of cocoons found		Number of fleas reared	Interval during which larva were observed	Mortality 100%
			Extremes	Aver.		Extremes	Aver.			
15 April '12	<i>X. cheopis</i>	32	46°	37°	.94	none	40	none	No larvae seen alive after 3 days	100
"	<i>P. irritans</i>	22	49°	37°	.93	"	41	"	"	100
"	<i>C. fasciatus</i>	22	49°	35°	.92	16*	April 39.3	"	"	No mortality during the course of experiment
			54°	35°	.95		May 39.3			
			50°	35°	.99		June 40.9			
			60°	37°	.95		July 43.6			

\* 16 Aug., 1 flea emerged; 6 Sept., 2 cocoons opened contained resting larvae; 9 Sept., 13 cocoons opened contained resting larvae.

TABLE LX.—Continued.

(c) Cocoons, spun by larvae in Incubator 75 Wet.									
Date	Species	Number	Temperature Extremes	Aver. Temp.	Humidity	Number of fleas which emerged	Aver. number of days in cocoon	Remainder of cocoons opened and found to contain	
15 April '12	<i>X. cheopis</i>	23	April 49° 35°	39.3	.92	mortality at least 96%	—	Two larvae came out of cocoons and died within the first few days. Remaining cocoons opened 26 July contained 20 dried up larvae and 1 living pupa (transferred to In- cubator 75 Wet, since died)	
"	<i>P. irritans</i>	18	May 54° 35°	39.3	.95			Three larvae came out of their cocoons, two died, one pu- pated. Remaining cocoons opened 26 July contained 2 dried up fleas, 2 dried up pupae, 11 dried up larvae	
"	<i>C. fasciatus</i>	22	June 50° 35°	40.9	.99	mortality 100%	—	Remaining cocoons transferred to cellar 26 July	
			July 60° 37°	43.6	.95				
Remaining cocoons opened 9 Sept. contained 5 resting larvae, 2 empty ("false" cocoons).									
(d) Adult fleas, newly emerged and unfed, kept in card jars with sand at bottom to burrow in.									
Date	Species	Number	Temperature Extremes	Aver. Temp.	Humidity	Length of life, days Minimum	Maximum	Average length of life, days	
29 April '12	<i>X. cheopis</i>	11	39.3	39.3	.95	7	36	20	
"	<i>P. irritans</i>	4	54° 35°	39.3	.95	7	38	16	
"	<i>C. fasciatus</i>	11		39.8	.96	7	51	34	

SUMMARY.

*Eggs and egg-laying.*

In comparison with the later stages in life history eggs are relatively insusceptible to external conditions. The range of variability in the length of time between laying and hatching, 2 to 10 days, is a small one and there is no appreciable tendency to utilize this stage for resting, the response to temperature changes being simple and direct.

The upper limit of temperature which is fatal to eggs has not been determined. *C. fasciatus* showed a high percentage hatching at 85° F. (see Table IV), while in the case of *P. irritans* 9 % hatched at 93° F. (see Table VI a), and on one occasion (Table VIII, series III) 27 % of the eggs of *X. cheopis* hatched at 93° F. At low temperatures the numbers which hatch in the case of all three species is reduced and cold is fatal to eggs of *P. irritans* and *X. cheopis*. In the ice chest, at an average temperature of 40·9° F. (see Table LX), 50 % of the eggs of *C. fasciatus* hatched, while all those of *X. cheopis* and *P. irritans* failed. A small proportion of eggs of the latter species hatched at 46° F., but in the neighbourhood of 55° F. appears to be the minimum for *X. cheopis*.

As regards the influence of humidity upon the hatching of eggs, in the range used in these experiments, only the larger differences produced consistent results, and, although there were some complete failures of hatching under conditions of low humidity, the evidence does not seem clear enough to warrant these being considered conclusive tests. On the whole, it is safe to say that a temperature of 65° to 80° F. with a humidity of ·70 or over is most favourable and that if the temperature be above 60° F. humidities below ·50 to ·55 are harmful. In the case of *P. irritans* there is possibility of complete failure at a humidity of ·50 but 70 % of the eggs of *C. fasciatus* hatched at a temperature of 75° F. with a humidity of only ·48 (see Tables II and IV).

Females of *C. fasciatus* show, in some cases, a fall in egg production under conditions of drought, but the effect is not noticeable with *P. irritans* and *X. cheopis*. Low temperatures check or prevent oviposition.

In the case of *C. fasciatus* and *P. irritans* warmth (75° F.) combined with low humidity, favours the fertility of eggs laid. This, in the case of *C. fasciatus*, might possibly be explained on the assumption that the fertility of ova is likely to be in inverse ratio to the number laid (see Table IV), but, in the case of *P. irritans* (Table VI) no such explanation is possible and we are forced to the conclusion that drought

is in some way, perhaps by acting on the instinct to copulate, responsible for an increase in the percentage of fertilized eggs. With *X. cheopis* drought does not seem to have any marked effect on the fertility of eggs (see Table VIII).

#### *Larvae.*

This stage may be subdivided into two periods, (1) an active and (2) a quiescent or resting phase, passed within the cocoon.

In the present work the second or resting period has, for convenience, been treated under a joint heading with the cocoon stage. For such treatment, there is some justification, for no genuine metamorphic stages could be more sharply separated from one another as regards power of resisting unfavourable conditions and death than are the periods of active and resting larval life respectively. The range of conditions which the active larvae are fitted to surmount is a narrow one, but, once within the cocoon, the species studied can easily tide over dangers that would mean 100 % mortality in the active stage.

Reference to Table XXV will show that in the dry incubators (75° F. and 84° F. and humidity .60) and warm cupboard (67°—69° F. and humidity .65—.71) active larvae of *P. irritans*, as well as of *C. fasciatus* and *X. cheopis*, died, while in the cocoon stage some survived. The fact that in these dry situations the majority of the insects which died in the cocoons were in the larval stage suggests that the transference of cocoons took place without allowing a sufficient interval of time to elapse after spinning.

In the case of all the species investigated, the newly hatched larvae were able to live from several days to over a month without food, provided the conditions were not otherwise unfavourable. Given food and reasonable conditions of temperature, the next important requirement is as high a percentage of moisture in the air as is compatible with surroundings so dry that there is no danger of a wet skin. Local moistening by the urination of animals or sweat from their bodies may convert what would otherwise be an impossible place into a favourable situation; while draughty conditions, with a comparatively high humidity, may be less favourable than a drier situation with a nearly still atmosphere<sup>1</sup>.

Evidence has been found of a difference in the kind of food necessary for *C. fasciatus* on the one hand and *P. irritans* and *X. cheopis* on the other. That the faeces of the adult fleas are a possible diet and

<sup>1</sup> This point may be of importance, as well ventilated houses will be far less suitable for flea-breeding than ill ventilated ones.

favourable for the larvae of all three species has been proved. At the same time there is some evidence which points to this food being a necessity for *C. fasciatus*. This divergence in the matter of food among larvae of the different species is consistent with the habits of the parents and the parental host.

The active larval period of *C. fasciatus* is not necessarily one of steady growth or continuous feeding, as larvae have been found from several days to two or three weeks after hatching without any appreciable increase in size. Even when full growth is attained a period of apparently unnecessary delay, or "lagging," sometimes occurs before the spinning of the cocoon is commenced (see Table XXVI). This mode of lengthening the larval stage probably occurs also with *P. irritans*, and possibly with *X. cheopis* as well.

The time occupied by the active larval period is subject to very wide variation, ranging from 15 to 114 days for *C. fasciatus* (Table XVI), 9 to 202 days for *P. irritans* (Table XXI), 12 to 84 days for *X. cheopis* (Table X), and 11 to 142 for *Ct. canis* (Table XXIV). Although low temperature is responsible for the instances of very protracted active life, the question of temperature is not the sole factor determining the length of the period elapsing between the hatching of the egg and the spinning of the cocoon, for marked individual variations occur in this respect between larvae from the same batch of eggs, reared under identical conditions.

#### *Cocoons.*

The duration of the cocoon period varied from 8 days to well over a year for *C. fasciatus* (Table XXVIII), from 7 to 239 days for *P. irritans* (Table XXXVIII), from 7 to 182 days for *X. cheopis* (Table XXXV), and from 7 to 354 days for *Ct. canis* (Table XL).

The question of how much of this period is spent as a larva, how much as a pupa and how much as a flea awaiting some stimulus to make it emerge, is no easy matter to determine. It is certain that *C. fasciatus* will rest for long periods as a larva—I have records up to 600 days. With the other species, however, the matter is far from being definitely settled. The observations indicate that the resting stage of *Ct. canis* is generally, if not always, imaginal. In the cases of *P. irritans* and *X. cheopis* the evidence is less definite but points to a rest in the imaginal state before emergence, when this is long delayed. I am of opinion, however, that a considerable period of the time elapsing between the spinning of cocoons and emergence is passed in the larval

condition. I have no evidence that the pupal period is ever utilized for resting. So far as can be ascertained, the development in the pupa, though delayed by cold and hastened by heat, is continuous until the pupal envelope is shed.

The length of the cocoon period is largely determined by conditions of temperature, but the range of variation, under exactly similar conditions, was found to be very considerable.

There is some evidence that a fall in temperature during larval life predisposes both *X. cheopis* (see Tables XXXIV and XXXV) and *P. irritans* (see pp. 537, 538) to lengthen their cocoon period. The results of the experiments with *C. fasciatus* (Table XXXII (b)) do not suggest that this happens in the case of this species. Table XXXIII, however, shows a predisposition on the part of November batches of larvae of this species to rest longer in the cocoon stage, apart from the actual condition of temperature obtaining at the time the cocoons were spun.

The cocoons of different species differ both in shape and texture, but the outward appearance is largely dependent upon the material in which the larvae live, fragments of which become attached during the spinning. The cocoons of *C. fasciatus* vary greatly as regards the texture and quality of the silk used, some being frail and loose, others as hard and brittle as though composed of glue rather than silk. There is good reason to conclude that hard cocoons are associated with lengthy resting periods (see pp. 610—612).

*Ct. canis* shows a strong disposition to spend the winter in the cocoon stage. In the case of *P. irritans* there is the same tendency, but it is less obvious, individuals continuing to emerge throughout the winter, if the weather is mild. *C. fasciatus* also frequently passes the cold weather in the cocoon, and a certain proportion of individuals of this species are in the habit of aestivating during the hot months and emerging during the cooler weather of autumn. In hot climates this habit might easily afford a basis for selective action and the consequent production of a race composed of individuals, displaying on the one hand a quick, and on the other greatly delayed, emergence without intermediate types. That this is a strongly ingrained habit is suggested by the emergence of individuals after periods of 100 to 150 days in the cocoon, at a temperature of from 75° F. to 85° F. Even at 93° to 95° F. a certain number of *C. fasciatus* are able to survive as resting larvae for periods of four months and then successfully complete their metamorphosis if removed to a lower temperature (p. 606).

Although with all the species the periods of feeding as larvae and of resting in cocoons are prolonged as the temperature falls, the optimum varies in the different species, being  $5^{\circ}$  higher for *P. irritans* and  $15^{\circ}$  to  $20^{\circ}$  higher for *X. cheopis* than for *C. fasciatus*.

Individual variation, as regards the time taken by these larval and cocoon stages, ensures the spreading of the adult emergence of rat and human fleas over a very wide interval of time. In the case of *Ct. canis* there would seem to be much more restriction for any one brood, although, with this species, some individuals of a brood emerging in autumn will, under suitable conditions, lie over the winter in their cocoons. It is evident that the protection afforded by the cocoon would be the means of saving this species from extermination, not only under conditions of drought (Table XLIV), but also under circumstances of excessive moisture, for it was proved that, in this stage, actual inundation could be survived for at least 12 hours.

#### Adults.

At  $45^{\circ}$ — $50^{\circ}$  F. with nearly saturated air, fleas can live for many days unfed; specimens of *P. irritans* have survived for 125 days, *C. fasciatus* for 95 days, *X. cheopis* for 38 days, *Ct. canis* for 58 and *C. gallinae* for 127 days (see Tables XLV, XLVIII and L). Yet under what might for insects be considered only moderately unfavourable conditions of temperature and humidity their powers of endurance are but slight in the absence of food. *X. cheopis* is little, if any, better fitted than *C. fasciatus* to withstand heat and drought in the absence of a host, but its habit of living on the rat rather than in its bed enables this species to extend the period of its active existence both in time and space and renders it a far more dangerous agent in the spread of plague.

Although kept in a box, if fed on their natural host, *P. irritans* may live for upwards of 513 days (Table LIV), *C. fasciatus* for 106 (Table LV) and *X. cheopis*, fed on man, for 100 (Table LVI). It is probable that both rat fleas, under natural circumstances, would live longer. *Ct. canis* and *C. gallinae* have lived for periods of 234 and 345 days respectively (Table LVII) when fed on man, and it is unlikely that they have a less lengthy life when fed on dogs or fowls.

In view of the suggestion that adult fleas imbibe fluids other than blood, some experimental tests were undertaken. Dissections were made, after an opportunity had been given the fleas to imbibe

coloured fluids, but no sign of the insects having fed could be traced. Among the observations on record, which suggest that adult fleas can be nourished otherwise than by warm-blooded animals, are those in which sick flies and Lepidopterous larvae are said to have been attacked<sup>1</sup>. I have made experiments with *C. fasciatus* and *Tenia fenestrella*. The results were negative and the fleas died as soon as the controls. The opinion I have formed as a result of my own experience is that feeding on warm blooded animals is essential to reproduction.

Most, if not all, the species dealt with have been observed to copulate shortly after emergence, but no signs of either eggs or brood have been observed in any of the boxes, jars, or other receptacles in which unfed fleas have been kept. Experiments conducted in specially prepared jars, containing food for any larvae that might result, and also abundant cover for the fleas, gave the same result. On the other hand, virgin females of *P. irritans*, when fed, laid freely, though only infertile eggs. In fact, during the course of these experiments, no support whatever has been obtained for the theory that flea breeding can take place from one generation to another in the absence of an animal host to provide food for the adult.

Although the several species of fleas other than human were successfully fed on man and lived for considerable periods, no eggs were laid by any of the species, save *C. gallinae*, when 15 minutes' daily feeding was permitted. Later attempts with the hair of the correct host placed in the boxes to afford cover, were also ineffective, but feeding for longer than 15 minutes daily, was sufficient to keep *P. irritans*

<sup>1</sup> The feeding by adult fleas on the larvae of Lepidoptera, noted by Boden (1882), seems to be just within the bounds of possibility, but is probably of rare occurrence. Most, if not all the Lepidopterous larvae, likely to be found in the same habitat as fleas, live in silken galleries, a habit which would afford great protection as the larvae are able to travel rapidly away from any spot where the silken tube may be pierced. The possibility of starving adult fleas attacking larvae of their own species has been specially considered as such a habit might lead to infection of the adult by any bacteria that might be living in the larval gut, and thus have an important bearing on the recrudescence of plague. I made the following trial: A number of unfed adult *C. fasciatus* were divided into two batches and placed in similar tubes and into one only of the tubes a number of flea larvae were placed. The length of life of the fleas in the tube containing the larvae was no longer than that of those in the control, while the larvae remained healthy and spun their cocoons as usual. The constant attention demanded by other work prevented any very close watch being kept on the activities of the fleas and I cannot state if any attempts were made to feed on the larvae.

Russell (1913) makes mention of attacks made by fleas on flies incapable of flight. It seems, however, improbable that this or any other similar food can be a serious factor in tiding the species over periods when no warm blooded hosts are available.



laying freely and fertile eggs were also obtained from *C. fasciatus* and *X. cheopis*. *Ct. canis* required to be given opportunity to feed for from 5 to 12 hours' daily to induce egg-laying. While *X. cheopis* feeds on man more readily than *C. fasciatus*, the females do not lay so freely, presumably because, in accordance with this species' practice of living upon its host, rather than in its nest, they require more frequent feeding than *C. fasciatus*.

The experiments in which the opportunities offered to adults of *P. irritans* to feed were varied (see p. 641) afforded no evidence that the amount of food taken had any influence on the *fertility* of the eggs laid, but only upon the *number*. This result confirms those obtained when rat and dog fleas were fed on a human host (see p. 479), and shows the great influence upon egg-production of the quantity of food taken by the adult. At the same time it further emphasises the improbability of breeding by unfed fleas.

It has been proved conclusively (p. 637) that male fleas are able to impregnate at least 13 females, and further that the females must pair more than once if the large number of eggs they are able to produce are to be of any service to the species.

It is uncertain whether the individuals that rapidly attain the imaginal stage are long-lived as adults, but it has been proved with regard to *P. irritans* that a long cocoon stage may be followed by a very long life as an adult.

My observations on the maximum duration of the various stages in the life history of *C. fasciatus*, *X. cheopis*, *P. irritans*, *Ct. canis* and *C. gallinae* may be summed up as follows:

*C. fasciatus*—egg stage 10 days, larval 114, cocoon 450, adult when fed 106, unfed 95.

*X. cheopis*—egg stage 10 days, larval 84, cocoon 182, adult when fed 100, unfed 38.

*P. irritans*—egg stage 12 days, larval 202, cocoon 239, adult when fed 513, unfed 125.

*Ct. canis*—egg stage 8 days, larval 142, cocoon 354, adult when fed 234, unfed 58.

*C. gallinae*—egg stage 7, larval (estimate) 50, cocoon (interrupted by opening) 70, adult when fed 354, unfed 127.

Adding together the maximum periods recorded for each stage, a fair indication of the possible length of life of the individual from egg until its death as a perfect insect is: for *C. fasciatus* 680 days, *X. cheopis* 376, *P. irritans* 966, *Ct. canis* 738, *C. gallinae* 481.

On this basis, and allowing for the longest recorded *unfed* imaginal lives, it will be seen that there is no difficulty in accounting for active adults being found, in favourable situations, where there have been *no hosts for considerable periods*. We may safely estimate for *C. fasciatus* 22 months, *P. irritans* 19 months, *X. cheopis* 10 months, *Ct. canis* 18 months, *C. gallinae* 12 months.

#### NOTE.

It is a matter of regret to me that the valuable and interesting paper "Observations on Flea breeding (*X. cheopis*) in Poona" (Report LV of the Advisory Committee, Plague Supplement II, *Journal of Hygiene*, Vol. XI, pp. 300—325) was not available before I had finished the experimental work and the writing of this report.

Notwithstanding the fact that the methods and conditions applying to the two series of experiments were so diverse, the great importance of humidity in controlling egg laying and hatching, the rearing of larvae and the length of the adult life is demonstrated alike by the Poona experiments and those of the present research which were carried out at Loughton, Essex. The Loughton experiments show, in addition, that both high and low temperatures are detrimental to *X. cheopis*, cold being especially fatal to the immature stages, a fact that the equable temperature of Poona tends to conceal.

The striking divergences in detail of the two sets of experiments are as follows, and must, I think, be attributed to the causes given below:

1. *Egg laying*. The much smaller number of eggs per female laid in Loughton must be attributed to the comparatively low temperature at which the cages were kept.

2. *Egg hatching*. The comparison is between ova obtained from excited insects struggling for foothold at Poona and those of individuals more comfortable, if less vigorous owing to the lower temperature, at Loughton. Under the most favourable circumstances only 49.1% hatched at Poona while the best Loughton record is 76%.

3. The greater larval mortality and shorter adult life at Poona is probably due partly to the use of methods less favourable to the insects than those employed at Loughton, and partly to a probable greater daily fluctuation of humidity at Poona.

In conclusion, I desire to record my thanks to many friends for their assistance and advice. I wish also to record my indebtedness to my assistant Mr H. J. Turner, for his valuable help and the care with which he entered up the records.

A. W. B.

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## DESCRIPTION OF PLATES XXVII—XXXIV.

Plates XXVII—XXIX, photographs of various fleas magnified 30 diameters. (The specimens are treated with hot 20 % caustic potash for a few minutes, dehydrated in alcohol, cleared in xylol and mounted in balsam.)

- Plate XXVII. Fig. 1. *Ceratophyllus fasciatus*, ♂.  
 „ 2. „ „ „ ♀.  
 „ 3. *Leptopsylla musculi*, ♂.  
 „ 4. „ „ „ ♀.

- Plate XXVIII. Fig. 1. *Pulex irritans*, ♂.  
 „ 2. „ „ „ ♀.  
 „ 3. *Xenopsylla cheopis*, ♂.  
 „ 4. „ „ „ ♀.

- Plate XXIX. Fig. 1. *Ctenocephalus canis*, ♂.  
 „ 2. „ „ „ ♀.  
 „ 3. „ „ *felis*, ♂.  
 „ 4. „ „ „ ♀.

- Plate XXX. Fig. 1. Ova, *Xenopsylla cheopis*, ×30.  
 „ 2. „ „ *Pulex irritans*, ×26.  
 „ 3. „ „ *Ceratophyllus fasciatus*, ×26.  
 „ 4. „ „ *Ctenocephalus canis*, ×30.

- Plate XXXI. Fig. 1. Ova, *Pulex irritans*, ×60.  
 „ 2. Skin of full-grown larva of *Ceratophyllus fasciatus*, ×17, showing the arrangement of dorsal plates and hairs.  
 „ 3. Head of *Xenopsylla cheopis*, ×90, showing the epipharynx and mandibles the elements which together form the piercing and sucking tube and beneath these the labium. The maxillary palps are in a drooping position.

- Plate XXXII. Living larvae, ×10.  
 Fig. 1. Dorsal, lateral and ventral aspects, *Xenopsylla cheopis*.  
 „ 2. Lateral aspect, *Ceratophyllus fasciatus*.  
 „ 3. In natural surroundings seen from above, *Xenopsylla cheopis*.  
 „ 4. Lateral aspect, *Pulex irritans*.

- Plate XXXIII. Fig. 1. Cocoons of *Xenopsylla cheopis*, ×10.  
 „ 2. „ „ *Pulex irritans*, ×10.  
 „ 3. „ „ *Ceratophyllus fasciatus*, ×10.  
 „ 4. „ „ *Leptopsylla musculi*, ×10.

- Plate XXXIV. Fig. 1. Pupae of *Xenopsylla cheopis*, ♂ and ♀, living, ×10.  
 „ 2. „ „ *Ctenocephalus canis*, ♀, living, ×16.  
 „ 3. „ „ *Leptopsylla musculi*, ♀, living, ×16.  
 „ 4. Cocoons of *Ctenocephalus canis*, ×10.



Fig. 1. *Ceratophyllus fasciatus* ♂ × 30.

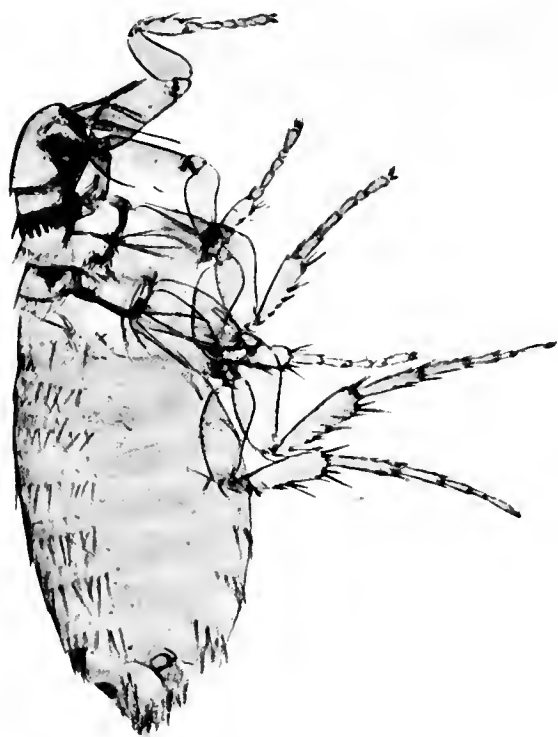


Fig. 2. *Ceratophyllus fasciatus* ♀ × 30.



Fig. 3. *Leptopsylla musculi* ♂ × 30.

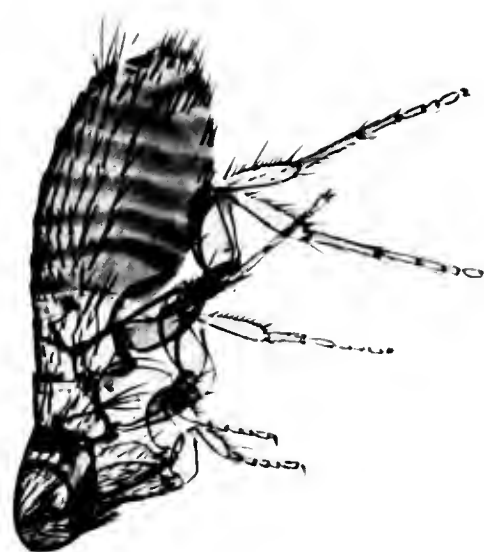


Fig. 4. *Leptopsylla musculi* ♀ × 30.

Photos by A. E. Tonge





Fig. 2. *Pulex irritans* ♀ × 30.

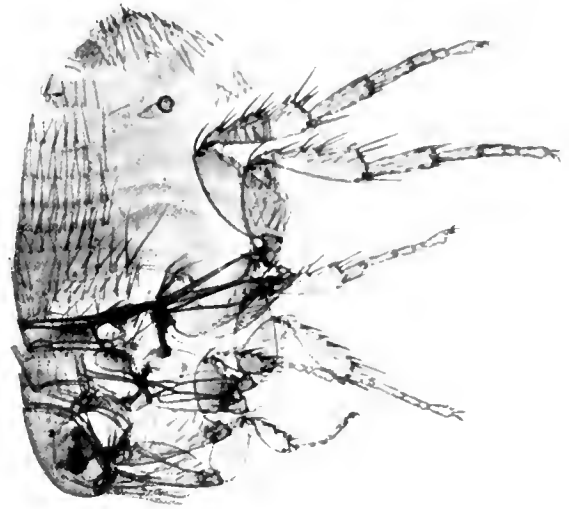


Fig. 4. *Xenopsylla cheopis* ♀ × 30.

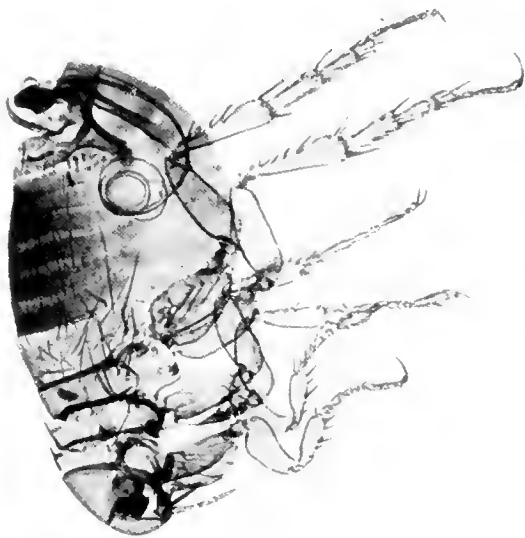


Fig. 1. *Pulex irritans* ♂ × 30.



Fig. 3. *Xenopsylla cheopis* ♂ × 30.

Photos by A. E. Tonge







Fig. 2. *Ctenocephalus canis* ♀ × 30.

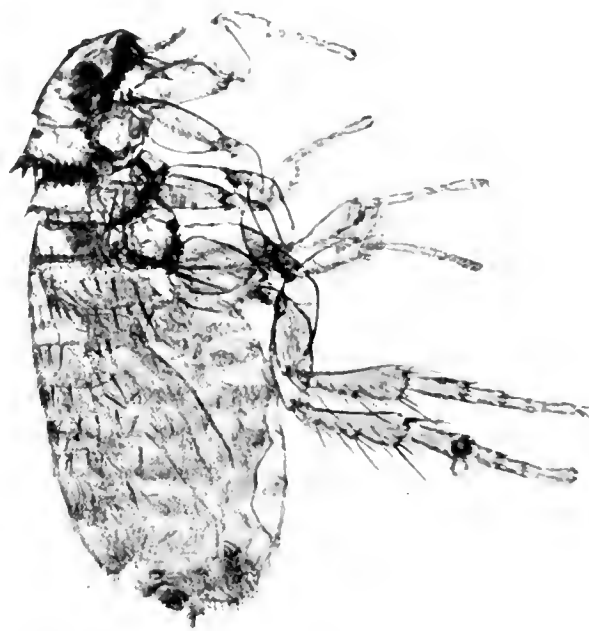


Fig. 4. *Ctenocephalus felis* ♀ × 30.

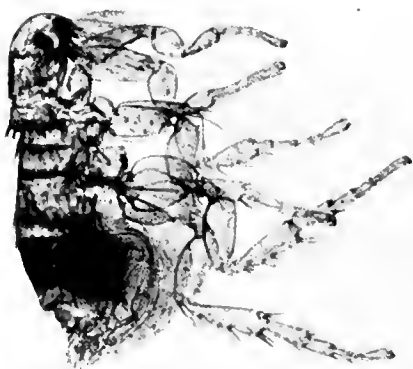


Fig. 1. *Ctenocephalus canis* ♂ × 30.



Fig. 3. *Ctenocephalus felis* ♂ × 30.

Photos by A. E. Tanga



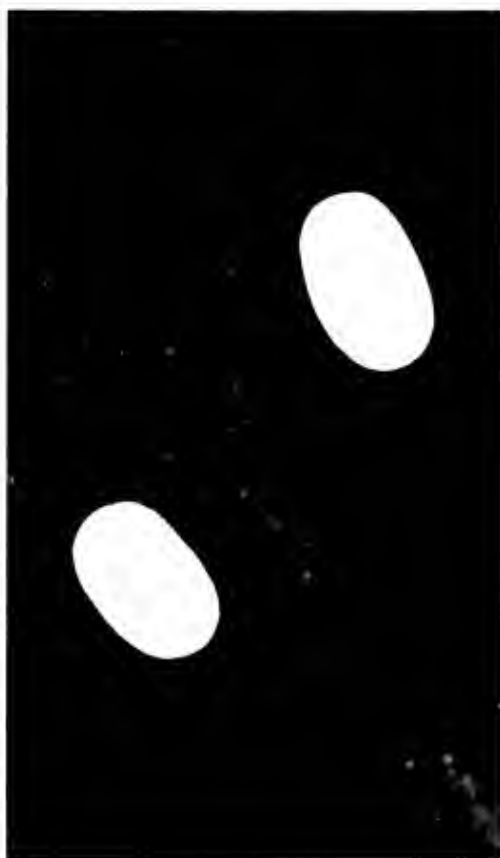


Fig. 2. Ova of *Pulex irritans*  $\times 26$ .



Fig. 4. Ova of *Ctenocephalus canis*  $\times 30$ .

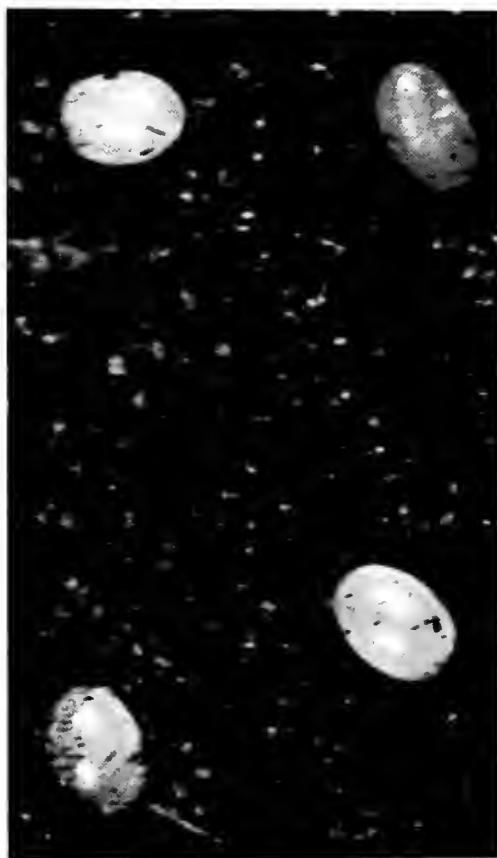


Fig. 1. Ova of *Xenopsylla cheopis*  $\times 30$ .



Fig. 3. Ova of *Ceratophyllus fasciatus*  $\times 26$ .



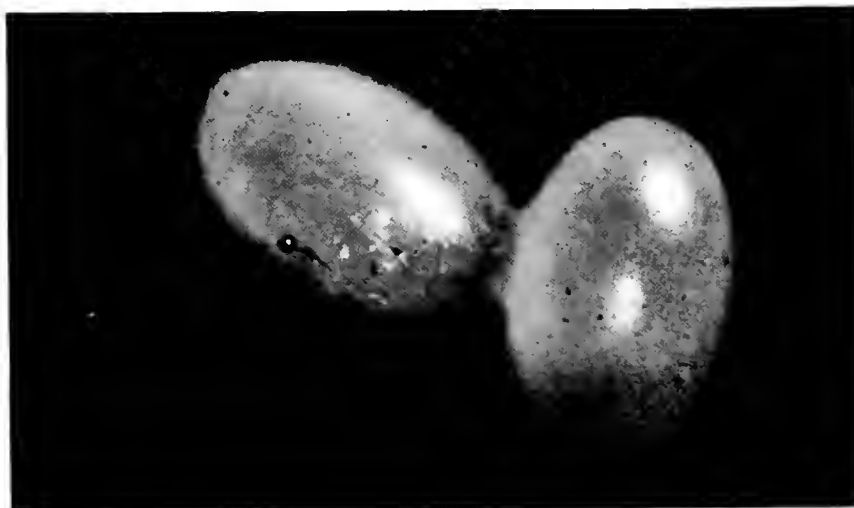


Fig. 1. Ova of *Pulex irritans*  $\times 60$ .



Fig. 2. Skin of full grown larva of *Ceratophyllus fasciatus*  $\times 17$ . Showing arrangement of dorsal plates and hairs.



Fig. 3. Head of *Neopsylla cheopis*  $\times 90$ .

Photos by A. E. Tonge





Fig. 1. Dorsal, lateral and ventral aspects of larvae of *Xenopsylla cheopis*  $\times 10$ .



Fig. 2. Larvae of *Ceratophyllus fasciatus*  $\times 10$ .



Fig. 3. Larva of *Xenopsylla cheopis* in natural surroundings  $\times 10$ .  
*Photos by A. E. Tonge*



Fig. 4. Larvae of *Pulex irritans*  $\times 10$ .





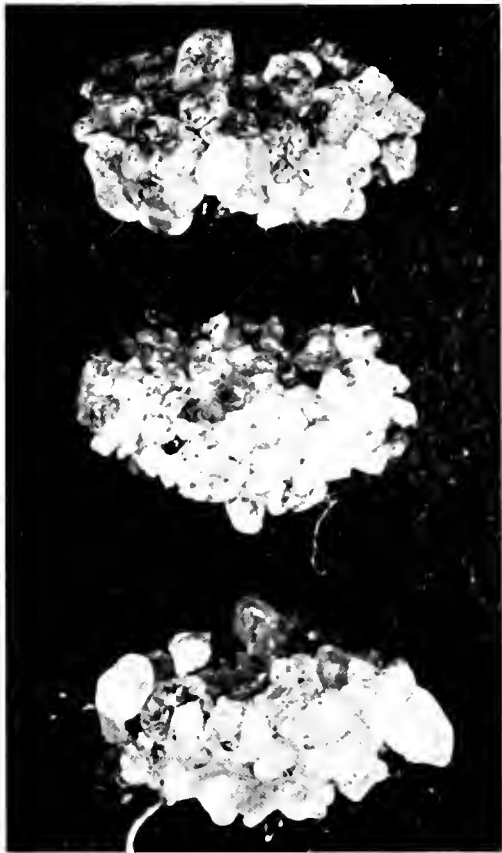


Fig. 2. Cocoons *Pulex irritans*  $\times 10$ .

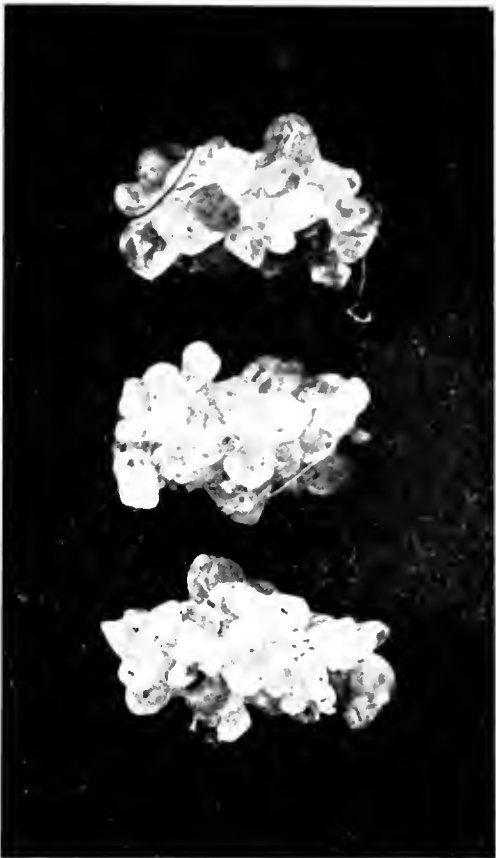


Fig. 4. Cocoons *Leptopsylla musculi*  $\times 10$ .

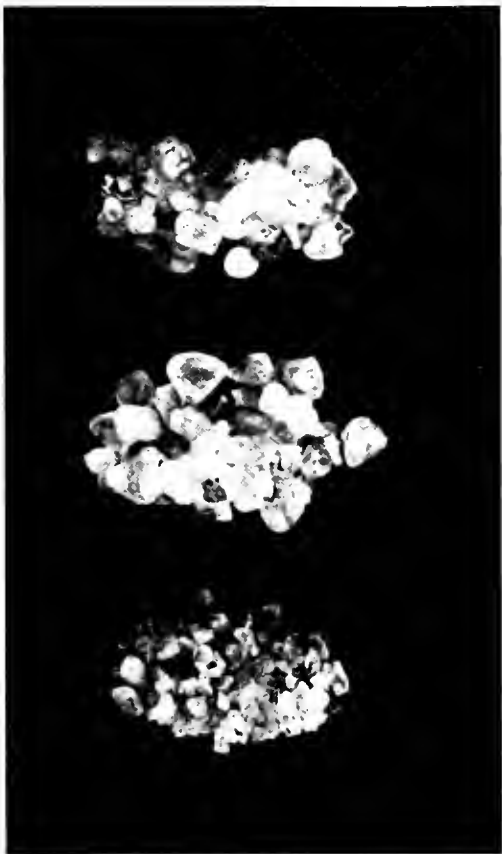


Fig. 1. Cocoons *Xenopsylla cheopis*  $\times 10$ .



Fig. 3. Cocoons *Ceratophyllus fasciatus*  $\times 10$ .

Photos by A. E. Ton, Jr.





Fig. 1. ♂ and ♀ pupae of *Xenopsylla cheopis*  $\times 10$ .



Fig. 2. Pupae of *Ctenocephalus canis*  $\times 16$ .



Fig. 3. Pupa of *Leptopsylla muscili*  $\times 16$ .

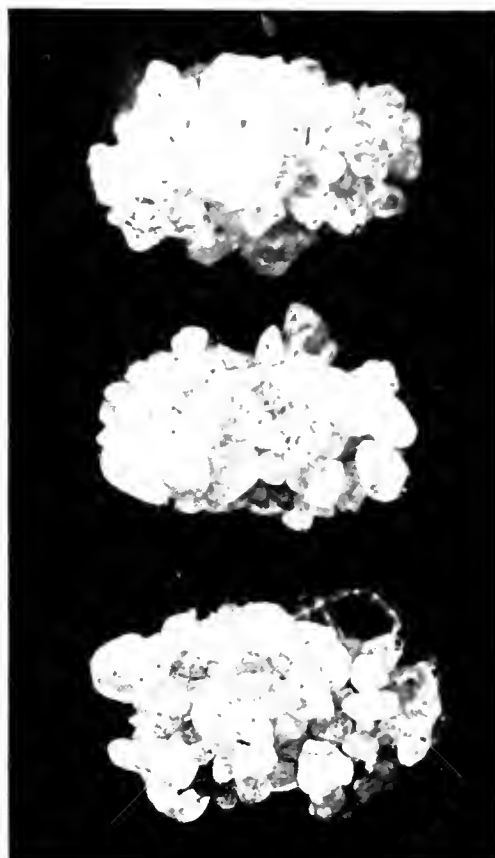


Fig. 4. Cocoons of *Ctenocephalus canis*  $\times 10$ .

Photos by A. E. Tonge



# LXX. ON THE SURVIVAL OF BACTERIA IN THE ALIMENTARY CANAL OF FLEAS DURING METAMORPHOSIS FROM LARVA TO ADULT.

BY A. W. BACOT, *Entomologist, Lister Institute.*

THE following experiments were undertaken to decide (a) if the gut of the flea-larvae may become infected with bacteria that are present in the food on which they are nourished, *e.g.* the faeces of their parents, and (b) if these organisms can survive within the gut during the metamorphosis from larva to pupa and pupa to imago.

## *Method of experiment.*

The fleas were artificially infected during the period of active larval life, and, in order to determine the power of the bacteria to survive in the gut, the fleas were afterwards examined for the presence of the infecting organism at the following stages in their life history, viz.

- (1) Active larvae.
- (2) Resting larvae, taken from cocoons.
- (3) Pupae, taken from cocoons.
- (4) Adult fleas reared from the infected larvae.

The infecting organisms used were :—*B. pyocyaneus*, *Staphylococcus aureus*, *Staphylococcus albus*, *B. enteritidis* (Gaertner), *B. violaceus* and *B. pestis*.

*Explanation of terms used.* *Active larvae*<sup>1</sup> signifies larvae that are or recently have been feeding and have not cleared the gut preliminary to spinning their cocoons.

*Resting larvae*<sup>1</sup> is used to designate larvae that have been taken from their cocoons and have cleared their guts preparatory to the change to the pupal state.

<sup>1</sup> These larval phases have been treated separately because it seemed of great importance for the purposes of this research to determine as nearly as possible the period of development at which the bacilli were got rid of, and further to discover, if possible, whether it was the clearance of the gut or the actual progress of histolysis which prevented the passage of the bacilli from the larval to the pupal stage.

*Pupae* signifies that the metamorphosis from the larval to the pupal stage has taken place, but covers a very wide range of development, from white or colourless objects that have only just rid themselves of the larval skin to fully developed fleas which are on the point of breaking out of the pupal envelope.

*Fleas or adults* had in every case emerged from the pupal envelope but in very many cases had not emerged from their cocoons.

*Methods of infecting the larvae.* Three methods were adopted. In the first the blood-soaked rag, used for feeding the larvae, was smeared with an emulsion of the infecting organism in sterile water. In the second, flies (*Musca domestica*) were allowed to infect themselves by taking food containing the bacillus used. The flea larvae were then permitted to feed upon flies which were killed and cut up, so that the larvae might obtain access to infected organs. In the third, the food supplied to the larvae consisted of strips of rag that had been soaked in the blood of a rat in the septicaemic stage after inoculation with Gaertner's bacillus. This last method was used at the suggestion of Dr A. E. Boycott, who very kindly supplied the infected food.

*Methods employed for sterilization.* It was not found possible to work under completely sterile conditions as far as the fleas were concerned. Tubes, food and the necessary sand for the flea larvae to crawl in were more or less easily sterilized, but it was not found practicable to sterilize the ova without killing them; only in one instance did an egg hatch after being sterilized. As regards the adults, the outward sterilization of such small insects in the imaginal stage presents many difficulties, as the external chitinous structure is very intricate, with many acute angles at the joints of limbs and their juncture with the body, in addition to the crevices afforded by overlapping plates, all of which afford possible shelter to such minute organisms as bacteria.

The method tried first was "flaming," passing the insects, after they had been chloroformed, one or more times through the flame of a Bunsen burner, either on a small platinum foil scoop, or on the point of a previously heated needle. The results were far from satisfactory, as it was impossible to subject any two insects to exactly similar conditions. The tendency to oscillate between a slow passage which left nothing but charred remains, and such rapid transit that the specimens were barely singed, led to such uncertain results that "flaming" was soon discarded in favour of immersion in solutions of lysol.

In the first series of experiments a 7 % or 10 % solution of lysol was used and, after immersion, the specimens were washed in sterile distilled water, then placed in a tube of broth, removed from this after 5 or 10 minutes and torn up in a second tube of broth with sterile needles. The first broth tube is called the control tube and the second the culture tube. After a series of tests the preliminary washing in distilled water was discarded, as it was found that the small quantity of lysol transferred with the flea did not prevent growth of the bacteria used in these experiments.

This method, although an advance on the attempts to sterilise the exterior of the insects by passing them through the flame, was not found to be sufficiently trustworthy, and it remained uncertain whether the bacterial growths obtained in many cases from adult fleas were not derived from the exterior of the insect (see experiment with *Staph. albus*, Table I).

The method finally adopted was the following. The insects reared from the infected larvae were first of all subjected to immersion in lysol to sterilize the exterior as far as possible, and after this had been done the alimentary canal was dissected out with very fine needles with the aid of a Zeiss Binocular dissecting microscope. The isolated organ was then subjected to bacteriological analysis. The necessity for this extra precaution in method is shown by the results of the following preliminary trials.

A number of cocoons of *C. fasciatus* were opened with sterile needles on sterilized slides, but they were not themselves subjected to any sterilizing process. In these cocoons 10 pupae were found, 1 resting larva and 1 cast larval skin. The 10 pupae were placed in separate tubes of broth; all produced a growth of a staphylococcus. The resting larva and the larval skin also gave a growth of staphylococcus.

A specimen of *C. fasciatus* taken from a rat was dissected with the precautions mentioned above and its stomach and gut placed in a tube of broth; a mould grew.

Three pupae of *C. fasciatus* were immersed for five minutes in 5 % lysol and then placed in tubes of broth, no growth resulted; after two days they were broken up in the tubes but the broth remained sterile.

Two pupae of *C. fasciatus* were dissected. In one the imaginal covering had already formed and the flea was extracted from the pupal envelope and dissected, its stomach and gut being placed in a tube of broth which remained sterile. The second pupa had not developed so

far and the imaginal skin had not formed. It was treated in the same way; no growth took place.

From these experiments it was judged that the pupae were certainly infected outwardly but that by immersion in lysol and subsequent dissection the danger of outward contamination might be avoided.

#### *Experimental results.*

In the earlier experiments, some of which are collected below in Table I, newly hatched larvae were infected with various organisms and subsequently entire larvae, pupae and adult fleas, after sterilization with lysol and washing in sterile broth but without dissection, were torn up in tubes of sterile broth to discover the presence of the infecting bacilli. Experiments in which the "control" tubes, *i.e.* those used for washing after sterilization, gave bacterial growth were discarded and are not included in the table.

From Table I it is evident that active larvae of *C. fasciatus* may become infected with *B. pyocyaneus*, when mixed with their food, and that this organism may survive in the larval resting period but does not persist to the pupal or adult stages. This result is confirmed in the experiment in which *B. enteritidis* was the infecting bacillus and larvae of *Xenopsylla cheopis* and *Pulex irritans* were employed in addition to those of *C. fasciatus*. In the experiment with *Staphylococcus albus*, growth of the infecting organism was not only recovered from a resting larva (*C. fasciatus*) but also from a pupa on one occasion and once from an adult flea. The choice of the organism in this case, however, was unfortunate, as *Staphylococcus albus* appears to be normally present and has frequently been isolated from the exterior of fleas and flea larvae. It is also significant that in the more careful experiments detailed below in which the alimentary canals of both pupae and the adult fleas were dissected out before being tested, they were found in every case to be sterile (see Table II). There is therefore a presumption that the imaginal and pupal growths originated from some outward infection that escaped the action of the sterilizing agents.

The method used for experiments in Table I was also adopted in a few experiments performed in conjunction with Dr Petrie, in which *B. pestis* was used as the infecting agent. The results were negative so far as the passage of the bacilli from larval to pupal or imaginal stages was concerned.



TABLE I. *Non-survival of bacteria in the alimentary canal of flea larvae during the subsequent stages in life-history. Infecting organism mixed with the food<sup>1</sup> offered to newly-hatched larvae.*

Species of flea	Infecting organism	Stage in life-history at which bacteriological analyses were made	No. of experiments	Result of tearing up whole insects in tubes of sterile broth after preliminary treatment with lysol and washing in broth
<i>C. fasciatus</i>	<i>B. pyocyaneus</i>	Active larva	2	Both gave growth containing <i>B. pyocyaneus</i>
		Resting larva	1	Growth containing <i>B. pyocyaneus</i>
		Pupa	6	2 sterile, 4 gave growth not containing <i>B. pyocyaneus</i>
		Adult flea	9	4 sterile, 5 gave growth not containing <i>B. pyocyaneus</i>
,,	<i>Staphylococcus albus</i>	Resting larva	1	Growth containing <i>Staph. albus</i>
		Pupa	2	1 sterile, 1 gave growth containing <i>Staph. albus</i>
		Adult flea	2	1 sterile, 1 gave growth containing <i>Staph. albus</i>
,,	<i>B. enteritidis</i> (Gärtner)	Active larva	5	2 sterile, 1 gave growth containing <i>B. enteritidis</i> , 2 growth not containing <i>B. enteritidis</i>
		Resting larva	4	2 sterile, 2 gave growth not containing <i>B. enteritidis</i>
		Pupa	4	2 sterile, 2 gave growth not containing <i>B. enteritidis</i>
		Adult flea	4	3 sterile, 1 gave growth not containing <i>B. enteritidis</i>
<i>X. cheopis</i>	,,	Active larva	1	Growth containing <i>B. enteritidis</i>
		Pupa	1	Sterile
		Adult flea	1	Sterile
<i>P. irritans</i>	,,	Active larva	1	Growth containing <i>B. enteritidis</i>
		Resting larva	2	1 sterile, 1 gave growth containing <i>B. enteritidis</i>
		Pupa	1	Sterile
		Adult flea	1	Sterile

<sup>1</sup> In some experiments with *B. pyocyaneus* the larvae were reared on a diet of dead flies which had been fed on material infected with the organism: in the experiments with Gärtner's bacillus the larvae were fed on blood taken from rats showing septicaemia due to this organism.

The following experiments were carried out at the Lister Institute in collaboration with my colleague Dr E. E. Atkin, who is responsible for the bacteriological part of the inquiry. In this work the alimentary canals of larvae, pupae and adult fleas were dissected out and tested separately, after the exterior of the insect had been subjected to a preliminary sterilization in lysol. In the set of experiments, which are detailed below in Table II, *Pulex irritans* and *Ceratophyllus fasciatus* were the species of fleas used and *Staphylococcus aureus* the infecting agent.

The following steps were taken to infect the larvae. Tubes containing the necessary allowance of sand and some pieces of lint were plugged with cotton wool and sterilized. Human blood, obtained under aseptic conditions, was mixed with an emulsion of *Staphylococcus aureus* and dropped on to the lint from a pipette. A number of larvae that had hatched in sterile tubes were introduced. Two tubes were prepared in this way; in one, experiments were carried out with larvae of *P. irritans* and in the other with larvae of *C. fasciatus*.

*P. irritans*. Experiment I. The tube, prepared as described above, was stocked with newly-hatched larvae; these were successfully reared, cocoons were spun and adult fleas emerged.

7 cocoons were opened and the pupae were dissected under sterile conditions after they had been soaked in 2% lysol; in some cases the fleas were extracted from the pupal envelope and removed to a fresh drop of sterilized water before dissection. The alimentary canals, as dissected, were transferred to tubes of broth. *All the tubes remained sterile.*

3 adult fleas were similarly treated, their stomachs and guts being transferred to tubes of broth. *All the tubes remained sterile.*

Experiment II. The same tube was re-stocked with newly-hatched larvae of *P. irritans*. These were reared and the alimentary canals subjected to bacteriological analysis at various stages as follows:—

(a) *Active larvae*. 3 larvae, in the active feeding stage, were taken from the infected tubes, immersed for 2 minutes in 2% lysol and dissected as before. In one case the isolated gut, in broth, produced a growth of *Staphylococcus albus*: in the second of *Staphylococcus albus* and *Staphylococcus aureus*: in the third, *Staphylococcus aureus* and trace of *Staphylococcus albus*. On another occasion, 5 active larvae were taken from the infected tube, immersed in 5% lysol for 5 minutes, dissected and treated as before. In 3 cases a growth of *Staphylococcus albus* was produced, in 2 cases the broth remained sterile. Finally, an

active larva was immersed in 3% lysol for 10 minutes. The isolated gut produced a growth of *Staphylococcus albus*.

(b) *Resting larvae and pupae.* 1 pupa and 1 resting larva were taken from the infected tube and dissected without previous immersion in lysol. The tubes of broth to which their alimentary canals were transferred remained sterile. 3 resting larvae and 4 pupae of *P. irritans* were immersed in 3% lysol for 5 or more minutes, their alimentary canals were then dissected and placed in tubes of broth. All remained sterile.

*C. fasciatus.* The tube, containing food infected with *Staphylococcus aureus*, used for this experiment had previously been stocked with *X. cheopis*, but the larvae were not successfully reared. It was then re-stocked with newly-hatched larvae of *C. fasciatus* and the experiment was successfully carried through.

(a) *Active larvae.* 1 larva, in the active feeding stage, was taken from the tube and immersed for 2 minutes in 2% lysol. The alimentary canal was dissected out and added to a tube of broth; growth of *Staphylococcus aureus* was obtained.

(b) *Resting larvae and pupae.* 3 resting larvae were taken from their cocoons, 1 was put into 3% lysol for 5 minutes, 1 for 15 and 1 for an hour, they were then dissected and the stomachs and guts placed in tubes of broth: 2 remained sterile, but the one that had been over an hour in the lysol developed a growth of *Staphylococcus albus*. On another occasion 3 cocoons were opened and 2 pupae and a resting larva were obtained. After immersion in 3% lysol for 5 minutes their guts and stomachs were dissected out and placed in tubes of broth. All remained sterile.

As a control to the above experiment, 3 cocoons of *C. fasciatus* from a tube that had not been artificially infected were opened and the contents examined. They contained 2 pupae and a resting larva. After soaking for from 10 to 15 minutes in 3% lysol, their stomachs and guts were dissected and placed in tubes of broth. All remained sterile.

*Experiments with B. violaceus.* In a further set of experiments an attempt was made to infect newly-hatched larvae of *C. fasciatus* with an organism isolated from water which gave a deep blue growth on agar and which was probably *B. violaceus*. The larvae were fed upon a blood-soaked rag wetted with an emulsion of the bacillus but no evidence was obtained that the larval gut was infected. 3 active larvae were examined and in addition 2 resting larvae and 7 pupae were taken

from their cocoons. All were placed in 3% lysol for 5 or more minutes, then washed in sterile water and dissected with the usual precautions. The tubes of broth containing the guts of two of the active larvae produced growths of *Staphylococcus albus*. In the case of the third, the tube remained sterile. In the case of the isolated alimentary tracts of the 7 pupae and 2 resting larvae all the tubes remained sterile. A further batch of 6 cocoons were opened and the 5 pupae and one resting larva they contained treated as before, save that the resting larva and two of the pupae remained in the lysol for more than 10 minutes. The broth inoculated with the pupal gut remained sterile but that containing the gut of the resting larva produced a growth of *Staphylococcus albus*.

TABLE II. *Non-survival of bacteria in the alimentary canals of flea larvae during subsequent stages in life-history. Infecting organism, Staphylococcus aureus, mixed with the food offered to newly-hatched larvae.*

Species of flea	Stage in life-history in which bacteriological examination was made	No. examined	Method adopted	Result in broth tubes to which the isolated gut was added
<i>P. irritans</i>	Active larva	9	Immersion in lysol, followed by dissection of alimentary canal	Sterile in 2 cases, growth of <i>Staphylococcus albus</i> in 5 cases, and growth of both <i>S. albus</i> and <i>S. aureus</i> in 2 cases
„	Resting larva	1	Dissection only	Sterile
„	„ „	3	Immersion in lysol, followed by dissection	All sterile
„	Pupa	1	Dissection only	Sterile
„	„	7	Immersion in lysol, followed by dissection	All sterile
„	Adult flea	3	„	All sterile
<i>C. fasciatus</i>	Active larva	1	„	Growth of <i>S. aureus</i>
„	Resting larva	4	„	3 sterile, growth of <i>S. albus</i> in 1 case
„	Pupa	2	„	Both sterile

*Summary of results and their significance.*

The results of the foregoing experiments show: (1) that the alimentary canal of the flea larva may become infected with the following bacteria if mixed with its food, viz.:—*B. pyocyaneus*, *B. enteritidis* (Gärtner), *St. albus* and *St. aureus*; (2) that an infection of the larval gut may persist until the resting period of the larva in the cocoon; (3) no satisfactory evidence that such infection can survive the pupal stage. No infection of the larval gut was demonstrated in the experiment with *B. violaceus*.

It has already been shown in another paper in this Supplement (Bacot, 1913) that flea larvae thrive on a diet composed of their parents' faeces and that for some species it is a normal, perhaps a necessary, source of food. *B. pestis* has frequently been detected in the droppings of *X. cheopis*, taken from plague-infected rats, by the Plague Commission (1908). I have confirmed this many times and also found the same to be true in the case of *C. fasciatus*. Verbitski (1906) states that *B. pestis* is to be found in the faeces of four different fleas, fed on animals suffering from plague. The conditions in the alimentary canals of flea larvae do not, however, appear to be very favourable to the growth of *B. pestis*. Larvae of *C. fasciatus*, taken from the bodies of mice dead from bubonic plague, whose hair was thickly speckled with the droppings of infected fleas, were dissected and smears made from the stomach contents. The number of cases, in which the microscopic examination gave a positive result, was very small and the bacilli, when present, were few and scattered. No trace was found of the massed multiplication which is so noticeable a feature in infected adult fleas (see Bacot and Martin, 1913).

An interesting contrast to the non-survival of bacteria in the fleas' gut after the larval stage is afforded by the Diptera, a group with which it is probable that the Siphonaptera have affinities. In the case of the house-fly (*Musca domestica*), infection of the alimentary canal at the larval period has been shown to survive to the adult stage by Bacot (1911), Ledingham (1911) and Graham-Smith (1911), with *Calliphora erythrocephala* also by Graham-Smith (1911) and with a species of *Sarcophaga* by Nicholls (1912).

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LXXI. THE EFFECT OF THE VAPOURS OF VARIOUS INSECTICIDES UPON FLEAS (*CERATOPHYLLUS FASCIATUS* AND *XENOPSYLLA CHEOPIS*) AT EACH STAGE IN THEIR LIFE-HISTORY AND UPON THE BED BUG (*CIMEX LECTULARIUS*) IN ITS LARVAL STAGE.

By A. W. BACOT, *Entomologist, Lister Institute.*

(With 1 Text-figure.)

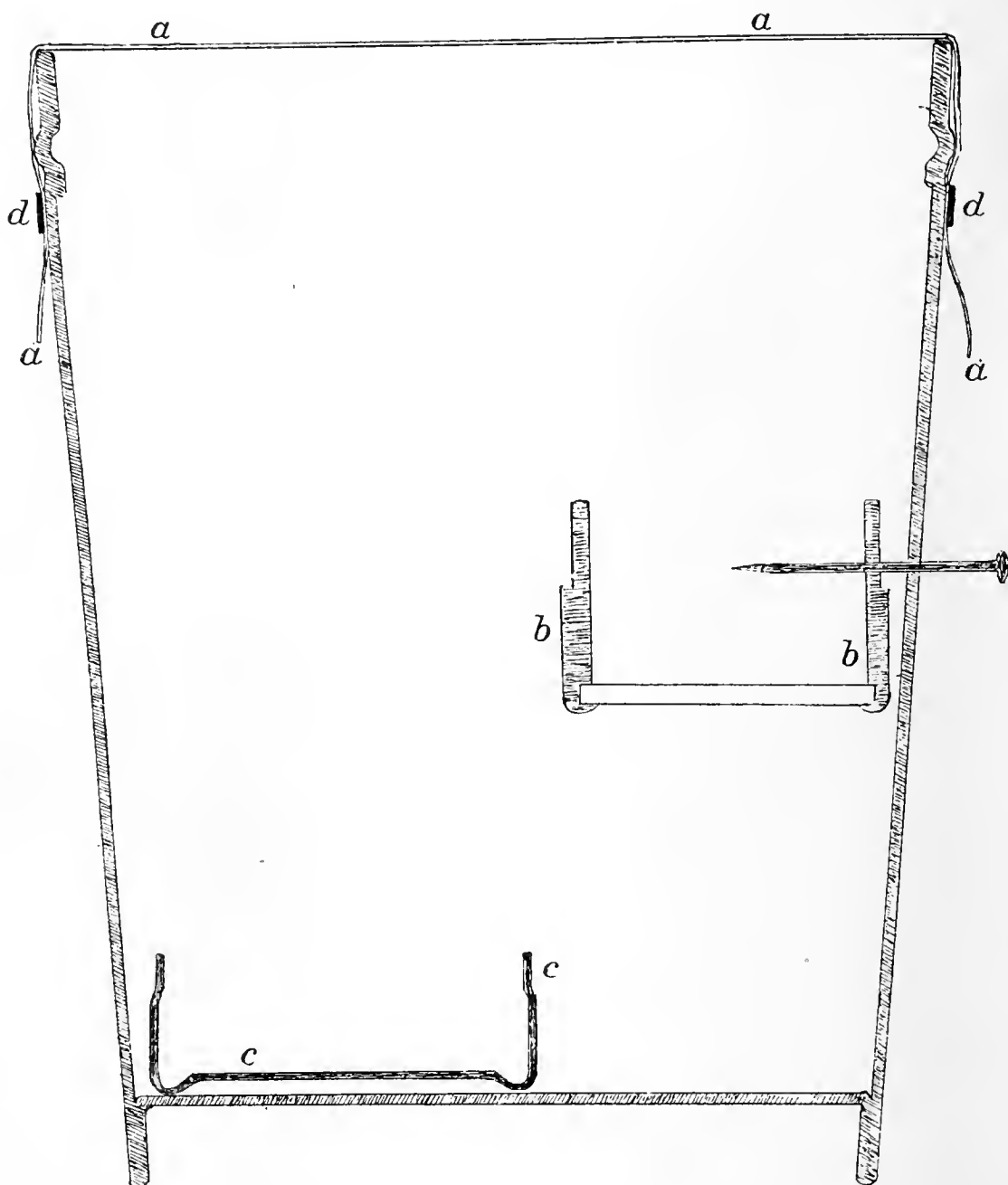
THE following experiments were carried out in order to test the relative efficiency of various insecticides and disinfecting fluids. No reasonable doubt exists as to the capability of the fluids used to destroy fleas in all stages, *provided actual contact takes place*, but, under the circumstances in which insecticides are commonly used, actual contact with all the individual fleas in any building in their various stages is wildly improbable. It is doubtful whether any *considerable* number would be killed if contact were a necessary condition. These experiments were accordingly planned with a view to testing the effectiveness of the vapour under conditions permitting free access of air.

*Method of experiment.*

The arrangement employed, shown in the diagram, Fig. 1, was the following. Half pint card cream-jars were the receptacles used (capacity = 330 c.c.). A small tin box without its lid was placed at the bottom to contain the insecticide, which, if liquid, was soaked up on several thicknesses of blotting paper. The fleas in larval, cocoon or adult stages as the case might be were placed in glass bottomed boxes one inch in diameter, either open at the top or with a covering of fine gauze. The box was fastened to the side of the jar by a pin about two inches above the bottom, so that contact with the insecticide was impossible and any vapour that reached the insects must have risen above the box containing them and have entered it from above.

In experiments with the egg stage, the ova were placed on a slip of blotting paper in glass tubes 3 inches high and  $\frac{3}{4}$  inch in diameter. The tube was uncorked and stood upright in the card jar, its base being kept from contact with the tin containing the insecticide. The jars

were covered with a piece of thin cotton cloth which would allow air to pass.



*a a a a.* Cover of thin cotton.

*b b.* Glass bottomed box in which the insects were placed.

*c c.* Open tin box containing the substance or liquid from which the vapour was given off.

*d d.* Elastic band.

The insecticides used were as follows: solutions of pure phenol, "lysol" (a proprietary article consisting of a mixture of cresols and soft soap), and formaline, commercial "benzine"<sup>1</sup> and paraffine oil,

<sup>1</sup> "Benzine" is the name given to a volatile petroleum oil much used in the arts.



flake naphthalene and crushed camphor. The fluid insecticides were renewed for each experiment, but the camphor and naphthalene served for several tests. The amount of liquid used at each trial was 0.5 c.c., and the weight of naphthalene and camphor about 0.5 grm., but as the solids evaporated slowly, the amount would be less than this for some experiments.

The trials are tabulated in order of the various stages in life history. All tests of any one stage were made at the same time from the same batch of ova, larvae, cocoons or fleas respectively. The comparative tests with the different insecticides were made under identical conditions, but the several stages and different species were not all tested at the same time. Therefore, although the tests are valid as between the different insecticides, they are not necessarily exactly comparable as regards conditions of temperature, humidity, etc. for the various insect stages.

The fleas used were the two common rat-fleas *Xenopsylla cheopis* and *Ceratophyllus fasciatus*; a few experiments were also made with the bed-bug *Cimex lectularius*.

In the experiments with *C. fasciatus* the temperature varied from about 50° F. to 60° F. With *X. cheopis* and *C. lectularius* the conditions as regards temperature and humidity are set out at the foot of each Table, on the whole the range of temperature was between 60° and 65° F. In my opinion the experiments with *C. fasciatus* are likely to afford the most reliable guide to any practicable application of the results so far as fleas are concerned. In considering the results obtained with *X. cheopis*, it is necessary to make some allowance for a general condition of low vitality which was manifest, due probably to the fact that the temperature (60°—65° F.) at which the experiments with eggs and larvae were performed, was a little too cool for this species which succeeds best at 70°—75° F. As regards experiments with eggs, I think a further allowance must be made for the unfavourable condition of temperature having an adverse influence upon the adults prior to their removal from the breeding cages for egg laying and causing an abnormal degree of infertility.

A further consideration to be kept in view is the possibility that temperatures higher than those at which these experiments were made might lead to more efficient action of the germicides. Although the general efficiency of all insecticides is likely to be increased at higher temperatures, yet the period of action of the more volatile ones would, on the other hand, be greatly curtailed. This becomes an important

point when it is remembered that probably both eggs and cocoons only display any degree of sensitiveness to insecticides at limited periods when rapid development is in progress.

*Experimental results.*

*C. fasciatus* (Tables I—VII). In making a comparative survey of the results obtained with *C. fasciatus* it has been found necessary to exclude Tables II and III, which give the results of some early experiments with larvae, and which are not quite comparable with the remainder of the series. The experiments detailed in Table III were discontinued, when the fluids had evaporated, because it was thought that all chance of their action must be at an end. In the light of the later trials, it appears that the vapours may act as a slow poison and that their action may not become apparent until 2 or 3 days after the complete evaporation of the fluid. As the larvae submitted to naphthalene were all active at the end of the first test (Table III) and the quantity of naphthalene had not apparently diminished, the jar in which this test took place was included in the new series (Table IV).

In examining the results, see Table VII, the action of the various vapours is seen to be markedly different at the different stages in life history, the larval stage being the most susceptible. For example with a 10% solution of "lysol," mortality in the egg stage was only 5%, whereas in the larval state it rose to 83%. In the cocoon and adult stages the figures obtaining were 50% and 25% mortality respectively (Tables V and VI). The action of formalin is very similar to that of "lysol" but is less potent, eggs and adult fleas proving quite insensitive to the vapour; larvae and cocoons suffered to the extent of 50% and 33% respectively. "Benzine" was ineffective especially in the egg and cocoon periods and paraffine oil was entirely without effect. Naphthalene and a 6% solution of phenol proved to be the two most powerful insecticides of those tried and of the two, naphthalene was the more efficient. The vapour of naphthalene proved deadly to all the individuals exposed, except in the egg stage where the mortality was 60%. In the case of phenol a few escaped at each stage.

*X. cheopis* (Tables VIII—XII). With this flea the results, as regards the effects shown upon the various stages in life history, are more erratic than was the case with *C. fasciatus*, and display no parallelism with those obtained with the latter species. This, however,

may be due to the early period at which the active larval and adult experiments had to be closed, owing to the fact that deaths were occurring among the controls. A study of the subsequent history of these experiments shows that the discrepancies are more apparent than real.

For *X. cheopis*, as with *C. fasciatus*, phenol in 6% solution and naphthalene proved most powerful agents. In both cases the adults succumbed entirely to their vapours, 100% being killed, and in the case of phenol the same was true of the larvae. The two insecticides were equal in their effect upon eggs and cocoons, 64% of the former and 94% of the latter being destroyed.

It is noteworthy that with this species the cocoon stage should be so susceptible to the vapours of "lysol," benzine and paraffine oil. This possibly is due to its short rest as a dormant larva, and the consequent longer adult life spent within the cocoon. This is well illustrated in the experiments with "lysol," where the mortality among the eggs was 64% and 80% in the case of both cocoons and adults, while in the tests made upon the active larvae no deaths occurred up to the time when the first death occurred among the controls. Formalin, on the other hand, killed nearly half the eggs and active larvae exposed to its vapour but did not harm either cocoons or adults. In the experiments with "benzine" the mortality was also noticeably high in the larval stage and nil in the adult stage.

The vapour of paraffine oil, which is without effect upon *C. fasciatus* in all stages, has a marked influence upon *X. cheopis*, and, on an average, killed nearly half the individuals of all stages exposed to it. It may be that the difference in the temperature at which the experiments were made in the two cases (15° F. or even 20° F.) produced sufficient increase in the quantity of vapour to account for this difference in death rate.

With *X. cheopis*, camphor has also to be ranged among the more potent insecticides, its action being quite equal to that of naphthalene and 6% phenol. Unfortunately we only have records for *C. fasciatus* in the egg and adult stages, but for these its toxic power appears to be insignificant. It may be that the difference is to be attributed to an enhanced evaporation in the experiments with *X. cheopis*, due to the higher temperature at which the experiments were made.

*Cimex lectularius* (Table XIII). For purposes of comparison, batches of *Cimex lectularius* in their second or third larval instars were tested at the same time as the larvae of *X. cheopis*.

It is doubtful whether in comparison of fleas with bugs the larval bugs ought not to be regarded as the equivalent of adult rather than larval fleas. As however the variations in resistance between the two insects proved so fundamental, the particular stage tested does not appear to be a question of great importance. The difference between bugs and fleas as regards susceptibility to the various vapours is quite remarkable. For bugs, phenol would appear to be much the most effective insecticide and naphthalene the least; the rapid action of the phenol is very significant in comparison with the other vapours. The discrepancy in the case of the larvae of bugs and of *X. cheopis* is even greater than at first sight appears, if the longer period that the bugs had to endure some of the vapours must be taken into account.

*Ammonia.* A single test was made with ammonia (specific gravity = .880), under similar conditions to those already described, 0.5 c.c. being the quantity used. A number of adult *C. fasciatus*, *X. cheopis* and *Cimex lectularius* were tested. The bugs were not affected at all. In one hour all the fleas were overcome. Six hours later some showed signs of recovery and after thirty-six hours several had quite recovered their activity and others were able to crawl, although they did not jump much. *C. fasciatus* appeared to stand the test better than *X. cheopis*, for all the fully active survivors were of the former species. The temperature during the experiment was about 55° F.

#### *Practical applications.*

In considering the practical application of the experiments described above, it is well to bear in mind that, as regards fleas, a quick remedy for getting rid of adults is not necessarily of service in permanently ridding a dwelling of fleas, and, at the same time, substances that are likely to kill the dormant individuals may be slower than is desired in their action upon the adults. For killing active *adult fleas or bugs* some liquid insecticide is much to be preferred and, whether crude phenol or petroleum be the basis of the insecticide, it is not possible to overestimate the importance of making it into an emulsion with soap, so that the contact with the insect may be secured. The vapours of both phenol and "lysol" solutions are also effective. They do not always kill speedily, but appear to act by affecting the intestinal tract, that at least is the impression gained from viewing the larvae that died when submitted to this test. This action is a great advantage and is not shared by paraffine oil.

"Benzine" acts speedily and makes sufficiently good contact without any addition of soap, but is uncertain as a vapour, and the insects in some cases recover after the "benzine" has evaporated.

For killing fleas in all stages, naphthalene, which is the most economical to use, seems also to be the most generally effective agent, owing, partly, to the slowness with which it evaporates. It is safe and clean to handle, and there is no restriction in regard to its sale to the public generally. These advantages it shares with camphor, but the latter suffers in comparison from its much greater costliness. For use in cracks or crevices naphthalene may be used as a liquid, either after being melted in a vessel plunged in hot water, or as a solution in "benzine." Paraffine oil is also a solvent, but not so good a one. A saturated solution in "benzine" can be poured into a crack or corner where it will solidify as the solvent evaporates. For use in rat-holes, especially in foundations, walls and the earth floors of plague-infected dwellings in hot countries, I am of opinion that a soap-carbolic or soap-petroleum emulsion (duly watered down) might be used with the addition of flake naphthalene. A few trials would determine the greatest quantity of naphthalene that could be stirred into the solution without impairing its fluidity. It could then be poured, or better pumped, into rat-holes. Probably a small portable spraying pump could be adapted for such service. By such a method both adult fleas and their brood would be satisfactorily destroyed.

A few experiments were made for the purpose of testing the effect of a strongly smelling ointment in preventing fleas and bugs from biting. A preparation was made in which a saturated solution of naphthalene in "benzine" was added to melted vaseline. This was used with and without the addition of clove oil which, however, was found to make but little difference. The ointment killed a number of *P. irritans* in a gauze covered box that was put face downwards upon a skin area smeared with the substance till the surface was shiny, but not coated thickly enough to rub off. A number of second instar *Cimex lectularius* in the same box did not feed, but were not otherwise affected. Insects of both species in control boxes on adjacent, but unprotected, skin areas fed freely. The death of the fleas was probably due to the benzine vapour which had not evaporated from the preparation. Subsequent tests with newly hatched bugs, in which 30 or 40 individuals were placed in each box, showed that the ointment was only a partial protection against the bugs. In one test 15 fed out of 38; in another, 16 fed out of 33. Controls showed 30 feeding out of 35 in one case and 44 out of 45 in another.

TABLE I. *Ova C. fasciatus*

A batch of eggs, divided into lots of 20, were placed on blotting paper in small glass tubes, and these were placed in the test jars on the 12 Nov. '11. All the ova were laid on the same day in the same box and the jars were kept under identical conditions.

Insecticide	No. of eggs that hatched	Aver. no. of days after laying	Percentage that hatched	Mortality, per cent.	Time elaps- ing, hours
Phenol (6 % solution)	13	11 days	65	35	432
"Lysol" (10 % solution)	12	13 "	60	40	
Formaline (10 % solution)	17	11 "	85	15	
Paraffine oil	16	12 "	80	20	
"Benzine"	15	12 "	75	25	
Naphthalene	Nil	—	Nil	100	
Camphor	15	14 "	75	25	
Control	13	13 "	65	35	

TABLE II. *Half-grown larvae C. fasciatus.*

32 larvae were taken from the flea-breeding cages; 20 submitted to naphthalene, 12 to camphor vapour.

Naphthalene, experiment commenced 3.15 p.m., 5 Nov. '11.

Date	No. of hours	
6 Nov.	17	all very sluggish
7 "	42	6 dead, 14 sluggish
8 "	67	18 " 2 "
9 "	90	all dead

Camphor, experiment commenced 10 a.m., 10 Nov. '11.

11 Nov.	24	all active
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No further examination made until the 17th, 168 hours, when all were dead.

TABLE III. *Larvae C. fasciatus*.

A batch of larvae were taken from the flea-breeding cages, 6 placed in each jar. All the jars were kept under identical conditions.

Date	Time elapsing, hours	Phenol about 3 0/0	"Lysol" 5 0/0	Formaline 5 0/0	"Benzine"	Paraffine oil	Naphthalene
29 Oct. '11	5	all active	all active	all active	all coiled 1 very sluggish when touched	all active	all active
	11	sluggish	1 quite still	"	all sluggish 1 quite still	"	all sluggish
	20	all active	5 very sluggish	"	1 apparently dead 3 very sluggish	"	very sluggish
30 Oct. '11	30	3 active 3 sluggish	5 active 1 dead	5 active 1 sluggish	5 active 1 dead	"	all very sluggish
	Mortality at 30 hours	nil	1 killed	nil	1 killed	nil	nil*

\* The naphthalene being still unexhausted and the larvae all living it was decided to continue this experiment and include it with the new series, see Table IV.

TABLE IV. *Larvae C. fasciatus*.

Date	Time elaps- ing, hours	Phenol 6%	"Lysol" 10%	Formaline 10%	Benzine	Paraffine oil	Naphthalene*	Control
31 Oct.	12	all active	all active	all active	1 no response to touch 2 feeble movement 3 sluggish	all active	3 apparently dead 3 sluggish	all active
	22	all sluggish	all very sluggish	all very sluggish	1 apparently dead 5 very sluggish	"	no change	"
1 Nov.	36	5 apparently dead 1 living	no change	no change	no change	"	5 dead 1 living	"
	46	5 dead 1 sluggish	2 apparently dead 4 living	2 apparently dead 4 sluggish	5 active 1 dead	"	1 "	"
2 "	60	no change	2 dead 4 sluggish	1 dead 1 nearly dead 4 sluggish	1 " 1 sluggish 4 active	"	1 very sluggish	"
	70	1 active	1 almost dead 3 sluggish	2 dead 4 sluggish	5 "	"	no change	"
3 "	84	"	3 dead	3 dead	5 "	"	"	1 has spun cocoon
	98	"	3 living 1 very feeble	3 living	2 dead 1 sluggish 3 active	"	"	no change
4 "	112	"	3 feeble	3 living	no change	"	remaining larvae very feeble	"
	122	clearing ready for spinning	another dead 2 very sluggish	3 active	3 have spun cocoons	"	no change	"
5 "	136	no change	only 1 living	3 " 1 has cleared ready to spin	3 cocoons 1 active	"	"	"
6 "	160	no change	no change	2 cocoons 1 active larva	as above	1 cocoon 5 active larvae	last living larva has died	3 cocoons 3 active larvae
Result at 160 hours	Mortality	83%	83%	50%	33%	nil	100%	nil

\* Continuation of previous experiment, the larvae had already been subjected to vapour for 32 hours.



TABLE V. *Cocoons C. fasciatus.*

29 Nov. '11. A number of recently spun cocoons were divided, a batch being placed in each jar.

Insecticide	No. of cocoons	No. of fleas that emerged	Result of examination of cocoons on 23 Dec. '11	Mortality, per cent.	Time elapsing, days
Phenol 6 %	6	1 on 6 Dec. '11	1 empty 1 contained dead pupa 4 „ „ larvae	83	24
“Lysol” 10 %	6	—	1 „ living larva 2 „ „ pupae 2 „ dead larvae 1 „ „ flea	50	24
Formaline 10 %	6	1 on 22 Dec. '11	1 empty 1 contained living pupa 2 „ „ fleas 1 „ dead pupa 1 „ „ larva	33	24
“Benzine”	6	1 „ 9 „	1 empty 2 contained living fleas 3 „ „ larvae	nil	24
Paraffine oil	6	1 „ 6 „ 2 „ 11 „	3 empty 3 contained living larvae	nil	24
Naphthalene	6	—	4 „ dead fleas 2 „ „ pupae	100	24

TABLE VI. *Adult fleas. C. fasciatus.*

Taken from the cages and divided into batches containing 4 males and 4 females, placed in jars 8 a.m. 2 Nov. '11; all jars kept under identical conditions.									
Date '11	Time elaps- ing, hours	Phenol 6%	"Lysol" 10%	Formaline 10%	"Benzine"	Paraffine oil	Naphthalene	Camphor	Control
2 Nov.	1	all active	all active	all active	not very active	all active	all active	all active	all active
3 "	12	2 feeble	"	"	1 active	"	1 active	7 active	"
	22	6 active	"	"	7 no movement	"	7 no movement	1 no movement	"
		1 dead	"	"	2 active	"	no change	1 dead	"
		2 feeble	"	"	6 no movement	"	7 active	7 active	"
4 "	36	1 dead	3 feeble	"	1 active	"	no movement	no change	"
		4 no movement	5 active	"	3 feeble	"			"
	46	3 active		"	4 no movement	"			"
		3 dead	5 feeble	"	4 active	"	6 apparently dead	1 feeble	"
		2 feeble	3 active	"	2 feeble	"	2 feeble movement	6 active	"
		3 active		"	2 dead	"			"
5 "	60	5 dead	1 dead	"	3 "	"	7 dead	7 "	"
		3 active	6 feeble	"	5 active	"	1 feeble movement		"
			1 active	"		"			"
6 "	84	no change	2 dead	"	3 dead	"	all dead	1 feeble	"
			6 feeble	"	1 no movement	"		6 active	"
				"	4 active	"			"
	94	"	3 dead	"	4 "	"	—	2 dead	1 dead
			5 active	"	1 feeble	"	100%	6 active	7 active
Mortality at 94 hours		62%	25%	nil	37%	nil		25%	—
Subsequent history.									
7 "	108	no change	1 feeble	all active	1 dead	2 dead	—	survivors active	2 dead
			4 active	2 eggs laid	4 active	1 feeble	—		1 feeble
						5 active	—		5 active
8 "	132	1 dead	1 dead	all active	3 dead	5 dead	—	2 dead	5 dead
		2 active	3 feeble		1 feeble	3 active	—	1 feeble	3 active
			1 active		1 active		—	3 active	
9 "	156	1 dead	2 dead	2 dead	4 dead	5 dead	—	4 dead	no change
		2 feeble	3 feeble	1 feeble	1 active	3 feeble	—	2 active	
				5 active			—		
10 "	180	2 dead	2 dead	3 dead	no change	6 dead	—	no change	5 dead
		1 feeble	2 feeble	5 active		1 feeble	—		3 active
			1 active			1 active	—		
11 "	240	no change	4 dead	6 dead	"	7 dead	—	4 dead	all dead
			1 feeble	2 active		1 feeble	—	2 feeble	

TABLE VII. *Summary of results. C. fasciatus. (Tables I, IV, V and VI.) Percentage mortality at different stages in life-history with various insecticides.*

*Note.* The experiment in the cocoon stage was not controlled; the mortality given is therefore the total. In the experiments with ova, larvae, and adult the mortality percent. is either reckoned before any deaths occurred in the control or, as in the case of the egg experiment, the mortality in the control is deducted before calculating.

	Phenol 6 %	"Lysol" 10 %	Formaline 10 %	"Benzine"	Paraffine oil	Naphtha- lene	Camphor	Control	Time elaps- ing, days
Ova	—	5 %	—	—	—	65 %	—	natural mor- tality 35 %	18
Larvae	83 %	83	50 %	33 %	nil	100 %	not tried	nil	6.6
Cocoons	83	50	33	nil	nil	100	,,	no control	24
Adults	62	25	nil	37	nil	100	25 %	nil	4
Totals	57	41	21	17	nil	91	12	—	—

*Note.* In the ova test the mortality was actually less than the control in the experiments with formaline, paraffine oil, benzine and camphor and equal to the control in the experiments with phenol.

TABLE VIII. *Ova X. cheopis.*

Batches of eggs were divided into lots (first experiment 10, second\* 15) and placed on blotting paper in small glass tubes, these being placed in the test jars. All the ova in the batch were laid on the same day. The jars were kept under identical conditions.

Insecticide	No. of eggs put into the tubes	No. which hatched	Percentage which hatched	Mortality, per cent.
Phenol (6 % solution)	10	nil	nil	100
	15	nil	nil	100
"Lysol" (10 % solution)	10	nil	nil	100
	15	nil	nil	100
Formaline (10 % solution)	10	4	40 %	60
	15	nil	nil	100
Paraffine oil	10	1	10	90
	15	1	7	93
"Benzine"	10	2	20	80
	15	1	7	93
Naphthalene	10	nil	nil	100
	15	nil	nil	100
Camphor	10	nil	nil	100
	15	1	7	93
Ammonia (s.g. .880)	15	1	7	93
Control	10	2	20	80
	15	14	93	7

Temperature about 60/65° F. Humidity uncertain.

\* *Note.* The experiment was repeated because of the small number which hatched on the first occasion.

TABLE IX. *Larvae X. cheopis*.

Larvae of various sizes were taken from the flea-breeding cages, divided into batches of 20 and placed in the jars at 11 a.m. on 22 June '12.  
All jars kept under identical conditions.

Date, 1912	Time elapsed, hours	Phenol 6%	"Lysol" 10%	Formaline 10%	"Benzine"	Paraffine oil	Naphthalene	Camphor	Control
23 June	24	6 dead 14 sluggish	all active	all active	16 dead 4 sluggish	2 dead 18 active	2 sluggish 18 active	7 dead 13 sluggish	all active
24 "	48	all dead	some started to spin	no change	1 dead 3 sluggish	6 dead 12 active	7 dead 11 active	6 dead 7 sluggish	no change
25 "	72	—	no change	9 dead 11 active	no change	6 " 6 started to spin	4 dead 4 active 3 sluggish	all dead	"
26 "	96	—	"	no change	2 sluggish 1 started to spin	6 active 1 started to spin	no change	—	some started to spin
Mortality at 96 hours		100%	nil	45%	85%	40%	55%	100%	nil

## Subsequent history.

27 "	120	—	7 active 5 sluggish 2 dead 6 started to spin	5 active 3 feeble 3 dead	2 feeble	5 active	7 sluggish	—	4 dead
28 "	144	—	3 active 2 dead 7 started to spin	no change	all dead	no change	1 dead 5 sluggish 1 started to spin	—	3 "
29 "	168	—	all dead	"	—	"	4 sluggish 1 started to spin	—	7 "
30 "	192	—	—	"	—	5 sluggish	all dead	—	6 living
			*(13 living)	(8 living)	*(1 living)	(7 living)	*(2 living)		

Temperature: max. 66.2° F., min. 60.5° F. Humidity 77.

\* In cocoons.

TABLE X. *Cocoons X. cheopis.*

10 July '12. A number of recently spun cocoons were divided, a batch being placed in each jar.

Insecticide	No. of cocoons	No. of fleas that emerged		Result of an examination of cocoons on the 10 Sept. '12	Mortality, per cent.	Time elapsing, days
Phenol (6 % solution)	15	—		15 dried up larvae	100	62
“Lysol” (10 % solution)	15	1 on	8 Aug. '12	11   ,,       ,,	86	62
		1	9 Sept. '12	2   ,,       pupae		
Formaline (10 % solution)	15	5	8 Aug. '12	*4 living fleas	nil	62
		2	19   ,,	1 empty		
		2	30   ,,			
		1	2 Sept. '12			
		*4	10   ,,			
“Benzine”	15	1	14 Aug. '12	*2 living fleas	73	62
		1	30   ,,	6 dried up pupae		
		*2	10 Sept. '12	1   ,,       larva		
				4 larvae came out of cocoons and died		
Paraffine oil	15	1	8 Aug. '12	2 living pupae	53	62
				2 resting larvae		
				8 dead pupae		
				2 empty		
Naphthalene	15	—		1 larva came out of cocoon and died	100	62
				13 dried up larvae		
				1   ,,       pupa		
Camphor	15	—		2 larvae came out of cocoons and died	100	62
				12 dried up larvae		
				1   ,,       pupa		
Control	15	4	8 Aug. '12	*9 living fleas	6	62
		*9	10 Sept. '12	1 dried up pupa		
				1 empty		

	Temperature		Humidity
	max.	min.	
July	68·5° F.	62·1° F.	·79
Aug.	63·3° F.	56·3° F.	·84

\* Taken from cocoon.

TABLE XI. *Adult fleas X. cheopis*.

Taken from the breeding cages and divided into batches of 10 (5 males and 5 females), put into boxes and placed in the test jars at 8 p.m. on the 11 July '12.

Date	Time elaps- ing, hours	Phenol 6%	"Lysol" 10%	Formaline 10%	"Benzine"	Paraffine oil	Naphthalene	Camphor	Control
12 July '12	12	all active	all active	all active	all active	1 dead 9 active	6 dead 4 feeble	all active	all active
13 "	36	all dead	2 feeble 8 dead	"	"	1 dead 8 active	all dead	3 dead 6 feeble	"
14 "	60	—	1 feeble 1 dead	1 dead 9 active	1 dead 9 active	4 dead 4 active	—	all dead	1 dead 9 active
Mortality at 60 hours		100%	80%	—	—	20%	100%	? 100%	—
Subsequent history.									
15 July '12	84	—	no change	1 dead 8 active	2 dead 7 active	4 active	—	—	9 active
16 "	108	—	all dead	no change	1 dead 6 active	1 dead 3 active	—	—	5 dead 4 active
17 "	132	—	—	3 dead 5 feeble	3 dead 2 active 1 feeble	2 dead 1 active	—	—	4 feeble
18 "	156	—	—	4 dead 1 feeble	2 dead 1 active	all dead	—	—	all dead

Temperature: max. 75.0° F., min. 66.5° F. Humidity, 78.

TABLE XII. Summary of Results. *X. cheopis*. Percentage mortality at different stages in life-history with various insecticides.

Note. The mortality percentage is either reckoned before any deaths occurred in the controls or the control mortality has been deducted from the totals.									
	Phenol 6%	"Lysol" 10%	Formaline 10%	"Benzine"	Paraffine oil	Naphthalene	Camphor	Controls	Time elaps- ing, days
Ova	64%	64%	48%	52%	56%	64%	60%	36%	4
Larva	100	nil	45	85	40	55	100	nil	62
Cocoon	94	80	nil	67	47	94	94	6	2.5
Flea	100	80	nil	nil	20	100	90	10	18
Totals	89	56	23	51	41	78	86	—	—

TABLE XIII. Larvae *Cimex lectularius*.

22 June '12. Batches of 10 larvae in 2nd and 3rd instars, kept in separate boxes in the same jars as the larvae of *X. cheopis*, the purpose being to test the relative resistance of fleas and bugs (see also Table IX).

Date	Time elaps- ing, days	Phenol 6%	"Lysol" 10%	Formaline 10%	"Benzine"	Paraffine oil	Naphthalene	Camphor	Control
*26 June	4	all dead	active	active	1 dead	2 dead	active	1 dead	active
27 "	5	—	"	2 dead	9 active	1 "	"	9 active	"
28 "	6	—	"	8 active	"	7 active	"	"	"
1 July	9	—	2 dead	"	"	"	1 dead	1 dead	"
10 "	18	—	6 "	1 dead	1 dead	"	9 active	2 "	"
Mortality after 180 days		100%	80%	30%	20%	30%	10%	40%	nil

Temperature: max. 65.6° F., min. 60.1° F. Humidity, 80.  
\* Date on which mortality of flea larvae (*X. cheopis*) in the corresponding experiment was reckoned.

182



## LXXII. EPIDEMIOLOGICAL OBSERVATIONS IN MADRAS PRESIDENCY.

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HAVENDRA RAO AND K. NARAYAN RAO.

(With 7 Maps and 30 Charts.)

### INTRODUCTORY.

It is well known that while many parts of India have suffered severely from plague, the Madras Presidency has been affected relatively slightly by the disease. An inquiry to throw light on this apparent immunity was undertaken by the Commission in the first instance in Madras City. The result of this preliminary inquiry is embodied in the Seventh Report of Plague Investigations in India published as a supplement to the *Journal of Hygiene*, 1912. These investigations in Madras City were directed to show (a) whether the conditions in that city as regards rats, fleas etc. were such as to be favourable or otherwise to the spread of plague, and (b) whether Madras has escaped plague because infection could not easily reach it.

As was perhaps to be expected in connection with an epidemiological inquiry of this kind, no single outstanding circumstance was discovered which could account for the freedom of the city from plague. On the one hand, rats and fleas were not so plentiful as in other plague-infected places investigated by us and perhaps so few as to render implantation of infection difficult, but there were probably sufficient numbers of both to enable infection to spread when once successfully implanted. On the other hand, the rats were found to be exceptionally susceptible to plague.

These facts offered no satisfactory explanation of the immunity of Madras to plague, so that it seemed likely that the city had escaped plague because infection had been unable to reach it, in spite of the

fact that considerable traffic exists between Madras and the badly plague-infected town of Bangalore.

Assuming that infection travels about the country for the most part in infected fleas associated either with merchandise, such as grain, or with the persons of human beings; it is clear that the likelihood of infection travelling any given distance will be proportional to the conditions being favourable to the life of the rat flea. Experiments available at the time this inquiry was set on foot had shown (Seventh Report of Plague Investigations in India, *Supplement to the Journal of Hygiene*, 1912, p. 317, see also Eighth Report, p. 613) that meteorological conditions have a very large influence on the duration of the life of rat fleas when separated from their normal host, a cool moist atmosphere allowing them to survive for about ten times as long as in hot dry air. Evidently, therefore, fleas would have some difficulty in arriving alive at any place which was surrounded by a zone of country where a high temperature, especially in conjunction with a low humidity, continuously prevailed. Madras City is, on the whole, a hot place, but though there is no definitely "cold weather" to afford really favourable conditions for flea importation, the temperature in the cooler months would apparently allow fleas to live for some considerable time. Taking these facts into consideration it seemed proper to extend the scope of the inquiry to other parts of the Madras Presidency, paying special attention to the conditions which influence the facilities for the importation of infection.

With this object in view, certain places were selected in which to establish small laboratories where investigations on rats and fleas were carried out on the lines adopted by the Commission in other places. Data were also collected bearing on the physical aspects and climate of the Presidency as well as on the facilities for communication between, and within, its various districts.

A study of the distribution of plague in the Madras Presidency, as will be shown later in this report, revealed the fact that, so far as the intensity of plague was concerned, this area could be divided into three more or less well-defined zones.

1. The Bellary district, the Mysore plateau and the Nilgiri Hills which had all suffered severely from plague ;
2. A zone lying immediately below and around the above-mentioned areas which had suffered but lightly from plague ;
3. The East Coast and Southern portion of the Presidency which had escaped the ravages of the disease.

Laboratories were therefore established in each of these zones. A large village called Denkanikota was selected as a suitable site for observations on the Mysore plateau. The towns of Coimbatore and Vaniambadi were chosen for observations in the zone below the Mysore plateau, while the town of Madura, with our previous observations in Madras City, represented the conditions in Southern India and on the East Coast respectively.

At these selected places observations were made, throughout a whole year, on the prevalence of rats and fleas, while visits, for the purpose of collecting data, extending for periods of about a fortnight at a time were paid to other places in the Presidency such as Ootacamund and Coonoor on the Nilgiri Hills, to Bellary, a town situated on a high lying plain to the north of the Mysore plateau, to the following towns on the West Coast: Mangalore, Calicut and Cochin; to Trichinopoly an inland town in the south of the Presidency; and to Cuddalore and Vizianagram on the East Coast, the former town being situated to the south of Madras in the district of the same name, while the latter is considerably further north in the district of Vizagapatam.

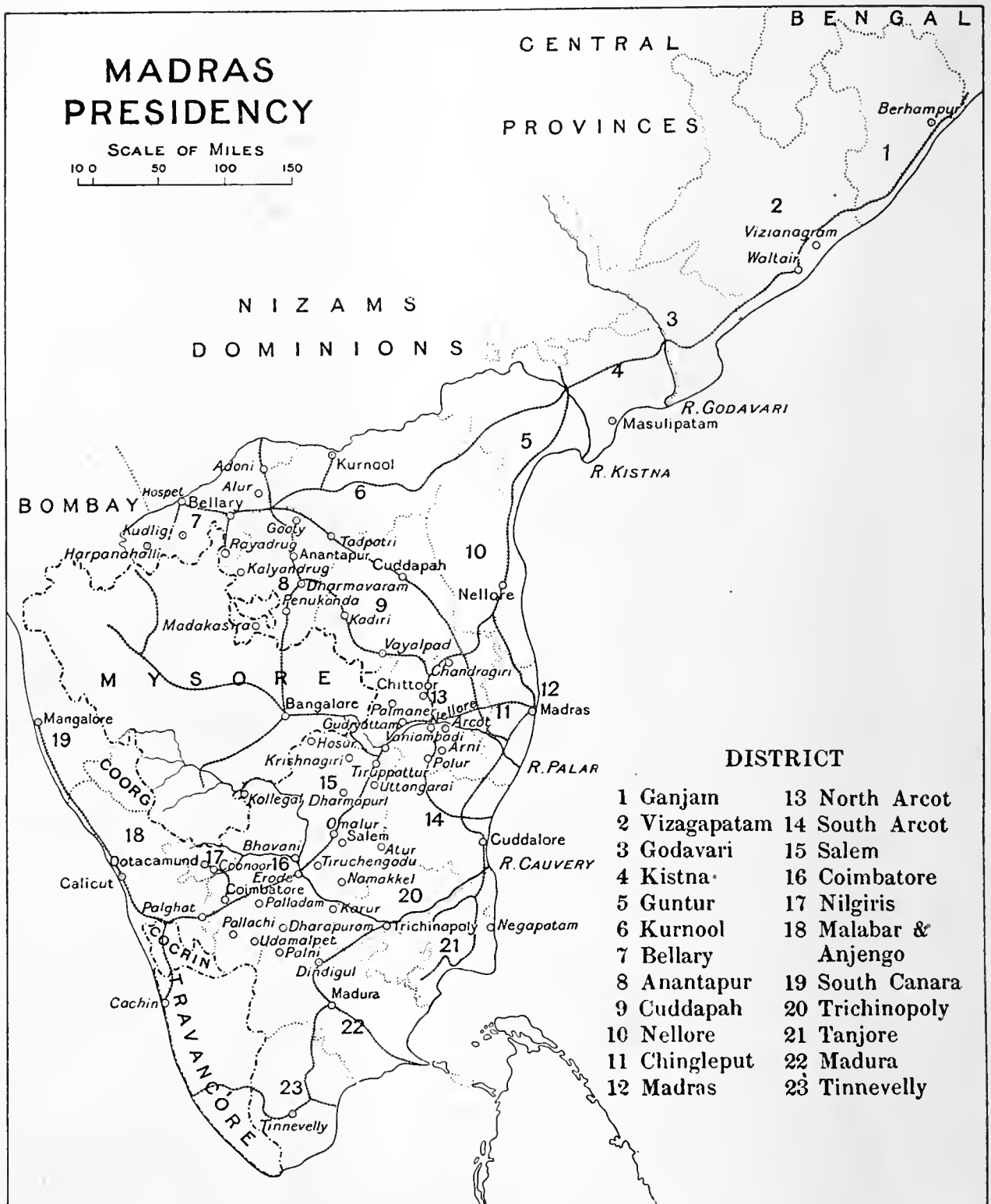
The information which has been collected in this way is considerable and the reader will perhaps bear with us while we attempt to clear the ground for a proper understanding of the data by describing in the first instance the administrative divisions of the Presidency; secondly, its general physical features; thirdly, its climatic conditions, and fourthly, the general distribution of plague in the Presidency, during the present pandemic, leaving for more detailed discussion the distribution of plague in certain of the more important infected districts.

### *I. Administrative divisions of Madras Presidency.*

For administrative purposes the Presidency is divided into 23 districts<sup>1</sup>. The districts have an average area of 7000 square miles and an average population of 1,879,000<sup>2</sup>. Each is divided into small areas called taluqs. For our purpose the districts may be conveniently divided into the following six main groups which are shown in Map No. 1:

<sup>1</sup> This was the division up to 1910, and, as all the plague figures, maps, etc. available for investigation have been compiled in accordance with this division we have found it necessary to keep to this arrangement for our purposes, although a few rearrangements have recently been made.

<sup>2</sup> All figures regarding population have been calculated on the 1901 census.



Map 1

(1) *The Northern Circars :*

Ganjam District.	}	These districts have all been free from plague.
Vizagapatam District.		
Godavari District.		
Kistna District.		
Guntur District.		

(2) *The Deccan or Ceded Districts :*

Kurnool District.	}	Bellary has been severely infected while the others have been affected to a lesser degree.
Bellary District.		
Anantapur District.		
Cuddapah District.		

(3) *The East Coast Districts :*

Chingleput District.	}	These districts have been practically free from plague.
Nellore District.		
South Arcot District.		
Tanjore District.		
Trichinopoly District.		
Madura District.		
Tinnevely District.		
Madras City.		

(4) *The Central Districts :*

North Arcot District.	}	All have been moderately infected with plague, especially Salem and Coimbatore.
Salem District.		
Coimbatore District.		

(5) *The West Coast Districts :*

South Canara District.	}	Localised epidemics have occurred in some of the larger towns of these districts.
Malabar District.		

(6) <i>The Nilgiris.</i>	}	The Nilgiris although showing few deaths has relatively to its population been severely affected.
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## II. *The physical features of the Madras Presidency.*

The physical conformation of the Presidency is mainly determined by the two chief hill ranges—the Eastern and Western Ghauts.

The Western Ghauts are a well-marked high range running along the western border of the Mysore State within about 50 miles of the sea, averaging 4000 feet in height, and with peaks rising in some cases as high as 8000 feet. These mountains are to a large extent covered with forest.

The Eastern Ghauts are a much less well-defined range, usually about 2000 feet high, frequently interrupted and much further from the sea. They first form a hill area in the jungle district of the Northern Circars, and are then interrupted for some distance by the

valleys of the Godavari and Kistna rivers; further south they are continued through the Guntur and Kurnool districts as the Nallamalai Hills. The valley of the Penner river then interrupts the continuity of the hills which still further south, however, rise as a series of ranges in the Central districts till they meet the Western Ghauts at the southern extremity of the Mysore State. At this spot, where the Eastern and Western Ghauts merge into one another, the elevated Nilgiri Hills are situated.

South of the Nilgiris there is a gap in the Western Ghauts, about 16 miles wide, called the Palghat Gap. Attention is drawn to this gap on account of the influence its existence has on the climatological features of the Coimbatore district. South of the Nilgiri Hills the Western Ghauts extend in a southerly direction along the western border of the Travancore State. This portion of the Western Ghauts is an elevated jungle-covered range to which, in different parts, a number of names have been given such as the Palni Hills, the Anamalai Hills, the Cardamom Hills.

Between the Western and Eastern Ghauts, as they converge to the Nilgiri Hills, lies the Mysore plateau, a high table-land at an elevation of from 2000 to 3000 feet. Most of this area is occupied by the Mysore State but small portions of the Madras Presidency, viz. the Hosur taluq of the Salem district and the whole of the Kollegal taluq and parts of the Satyamangalam and Bhavani taluqs of the Coimbatore district are also situated on this plateau. A somewhat lower-lying plateau, at a general elevation of about 1500 feet, extends between Mysore State and the Nizam Dominions. On this plateau is situated the whole of the Bellary district and parts of the Anantapur and Kurnool districts. These physical features are shown in Map 2.

In considering the effect of the physical conformation of the country on the distribution of plague, it is to be remembered that while some of the elevations shown on the map are high-lying flat ground, well populated and under cultivation, such as the Mysore plateau and the elevated plain on which the Bellary district is situated, other elevations represent definite hills, many of them covered by forest and very sparsely, if at all, inhabited.

### III. *Climatic conditions in the Madras Presidency.*

The climate of the Madras Presidency presents very varied features, for the Presidency extends over twelve degrees of northern latitude. It has a long coast line, and inland has low-lying as well as elevated



## 690 *Epidemiological Observations in Madras Presidency*

plains and hill and mountain ranges attaining in places altitudes of over 6000 feet. The effect of these physical features on the climate is still further influenced by monsoon currents which spread rain over separate areas at different times of the year. In these circumstances it is difficult to give any general idea of the climate of the Presidency as a whole. A careful study of the accompanying charts (figs. 1—21) which have been prepared from data furnished by the meteorological department, will, however, give the reader some idea of the climatic conditions in certain parts of the Presidency. These charts may be usefully compared with similar charts prepared for certain selected places in India where plague epidemics have been frequent. A word or two must be said regarding the construction of the charts. The figures on which they are based are the so-called "normal figures" used by the Meteorological Department. These "normal figures" we understand have generally been calculated from daily readings taken at the respective observatories for a period of about twenty-five years. The figures used in the construction of the charts are, as regards temperature, the average for a period of ten days of the mean normal temperature of the day; as regards relative humidity the average for the same period of the normal percentage humidity calculated on daily readings taken at 8 a.m. It must be clearly understood that these charts give only a somewhat imperfect idea of the climate of the places selected. In so far as they represent the normal conditions, they do not afford any idea of the variations from the normal in different years, and variations above and below the mean of the day are not illustrated. In these respects they are defective for the study of plague epidemics. It is noteworthy that in a place like Coimbatore, a town in the Madras Presidency where we have examined the relation of temperature and humidity to the plague epidemics which have visited it for a period of ten years, these epidemics as a rule occurred in abnormal years when the temperature was lower and the humidity higher than normal.

With these somewhat serious limitations the charts nevertheless serve a useful purpose in that they enable us to judge for how long a period and to what extent the mean daily temperature falls below 80° F., a temperature which we found was a very critical one for plague<sup>1</sup>. It has been shown that epidemics generally begin when the mean daily temperature lies between 60° and 80° F. and that they rapidly come to a close when the mean temperature rises above 85° F.

<sup>1</sup> Vide "An analysis of the influence of temperature in six plague-infected localities" in India which was published in the *Journal of Hygiene*, Vol. VIII, pp. 274—279.



The relation of humidity to temperature is also a matter of some importance, and a high relative humidity with a mean temperature well below 80° F. is favourable for plague epidemics<sup>1</sup>.

It will be convenient to examine the charts in the following order: East Coast stations (Charts 1—8), West Coast stations (Charts 9—11), Inland stations below 1000 feet elevation (Charts 12—15), Inland stations at elevations over 1000 feet (Charts 16—21).

Taking the East Coast stations first and beginning in the south with Negapatam in the Tanjore district (Chart 1) we will pass northwards to Cuddalore in South Arcot (Chart 2), thence to Madras (Chart 3), Nellore (Chart 4), Masulipatam (Chart 5) in the Kistna district, Coconada in the Godavari district (Chart 6), Waltair in Vizagapatam (Chart 7) and to Berhampore, the most northerly station, situated in Ganjam (Chart 8). An examination of these charts reveals the fact that the mean temperature in these places is about 80° F. and below this only from November to the beginning of March. It seldom falls below 76° F. in Nellore and the stations south of it, while from Masulipatam northwards the mean temperature falls as low or lower than 74° F. for a month or more in the winter. The relative humidity is high (over 80 %) for the whole of the cold season in Masulipatam and

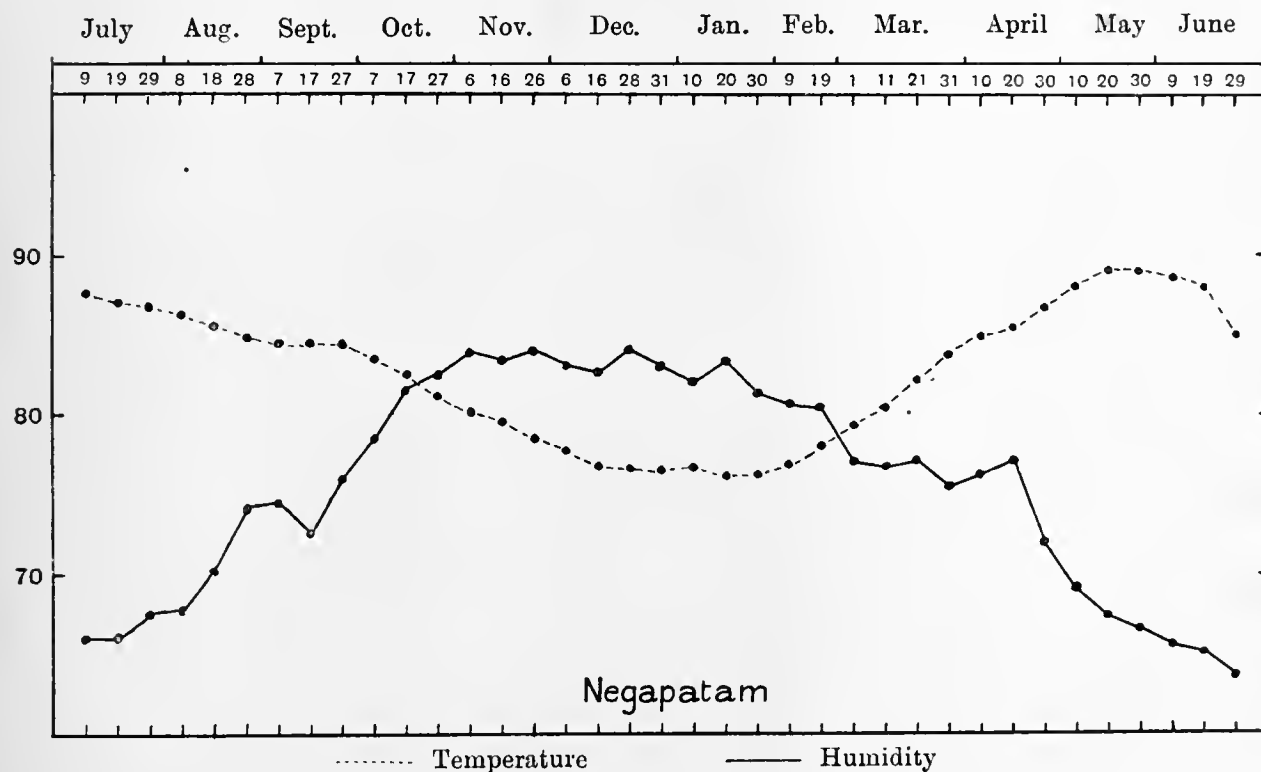
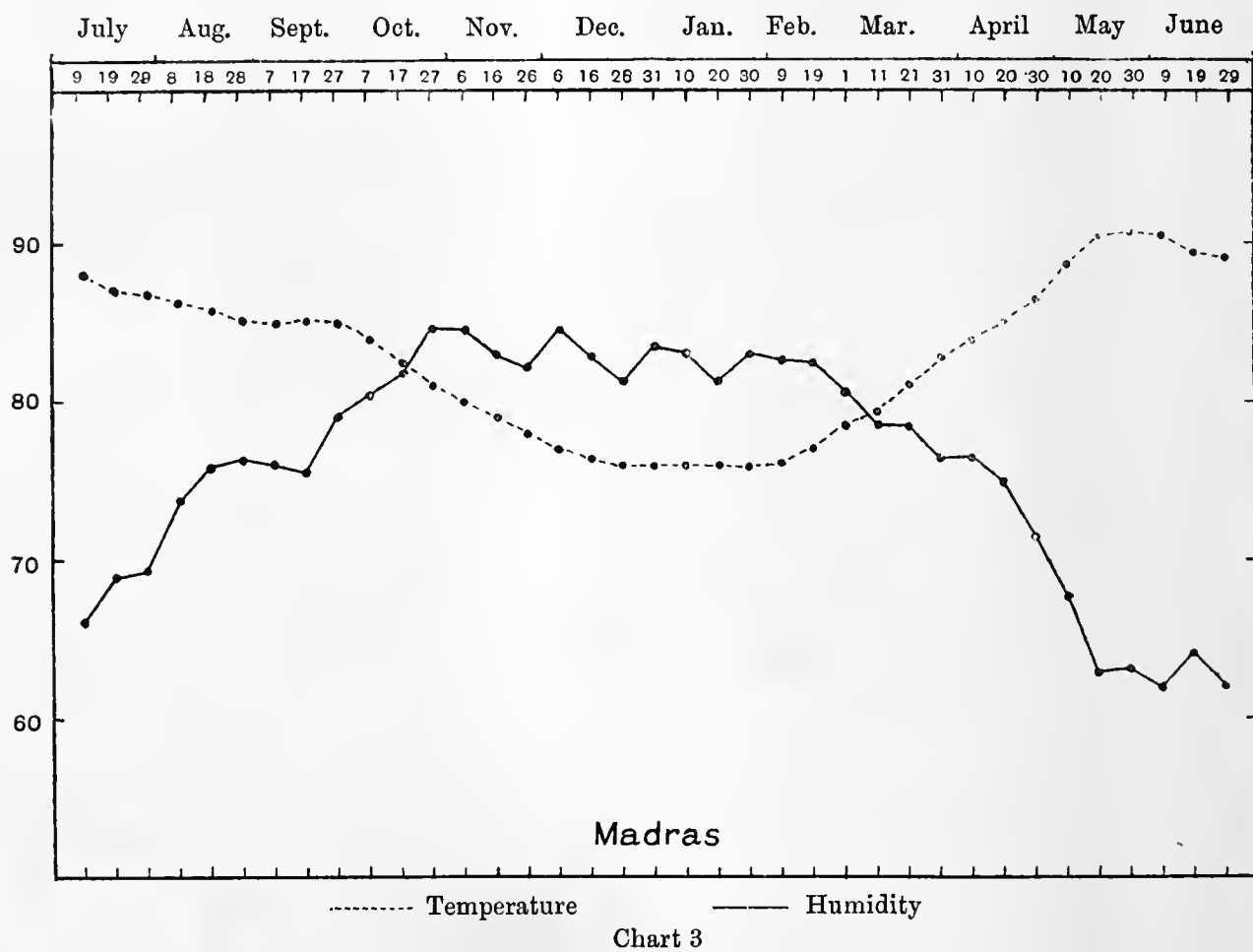
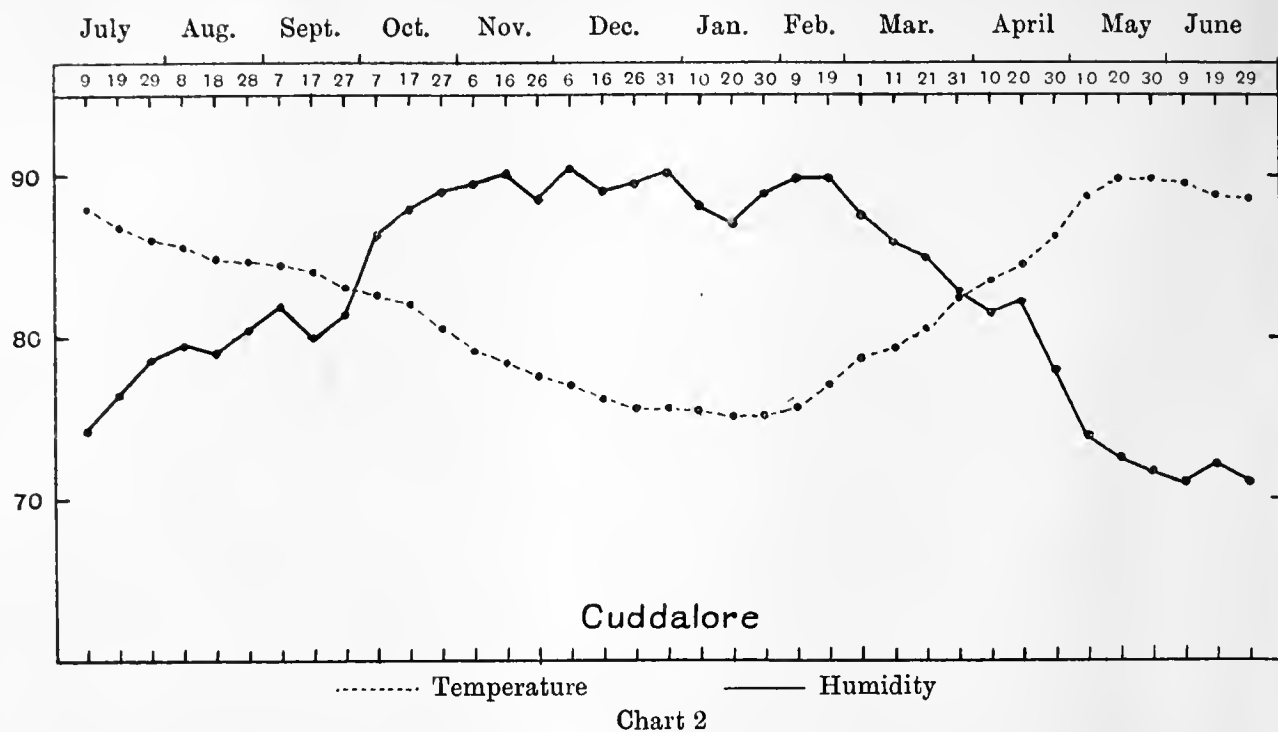


Chart 1

<sup>1</sup> See plague epidemics in the United Provinces.

692 *Epidemiological Observations in Madras Presidency*



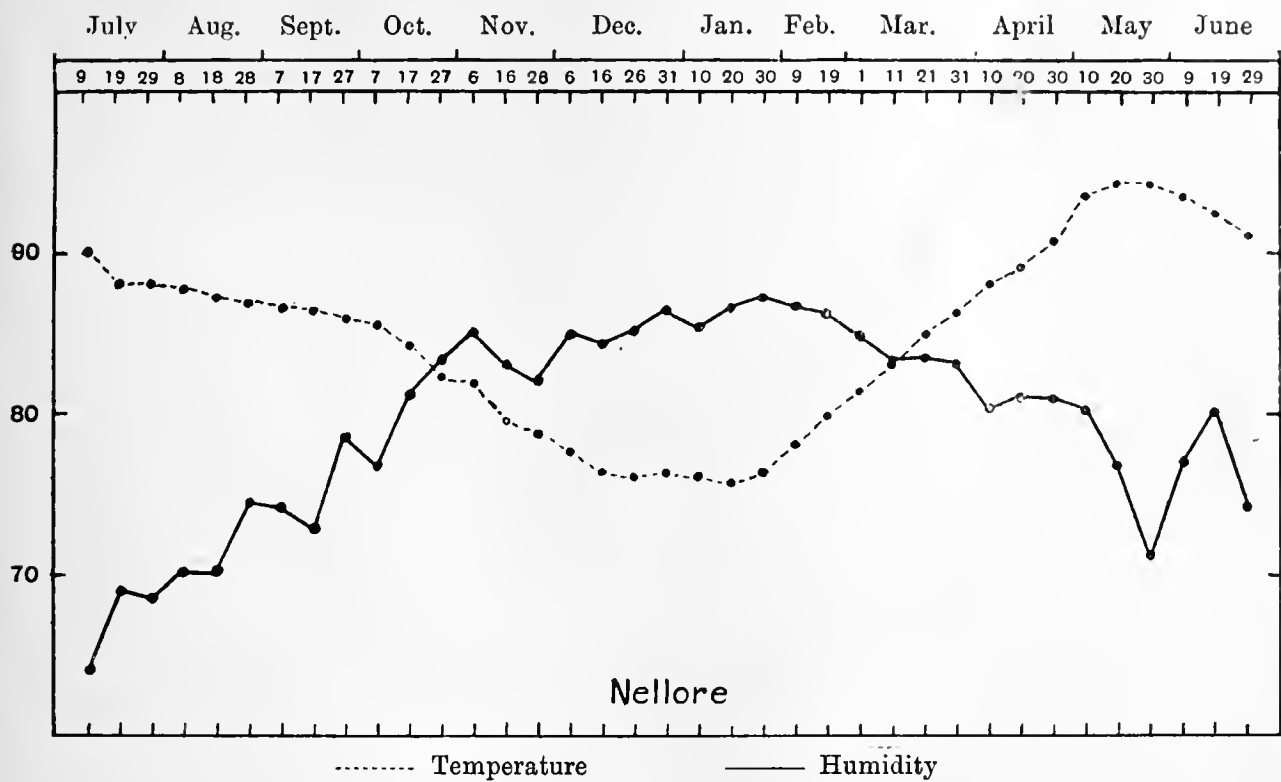


Chart 4

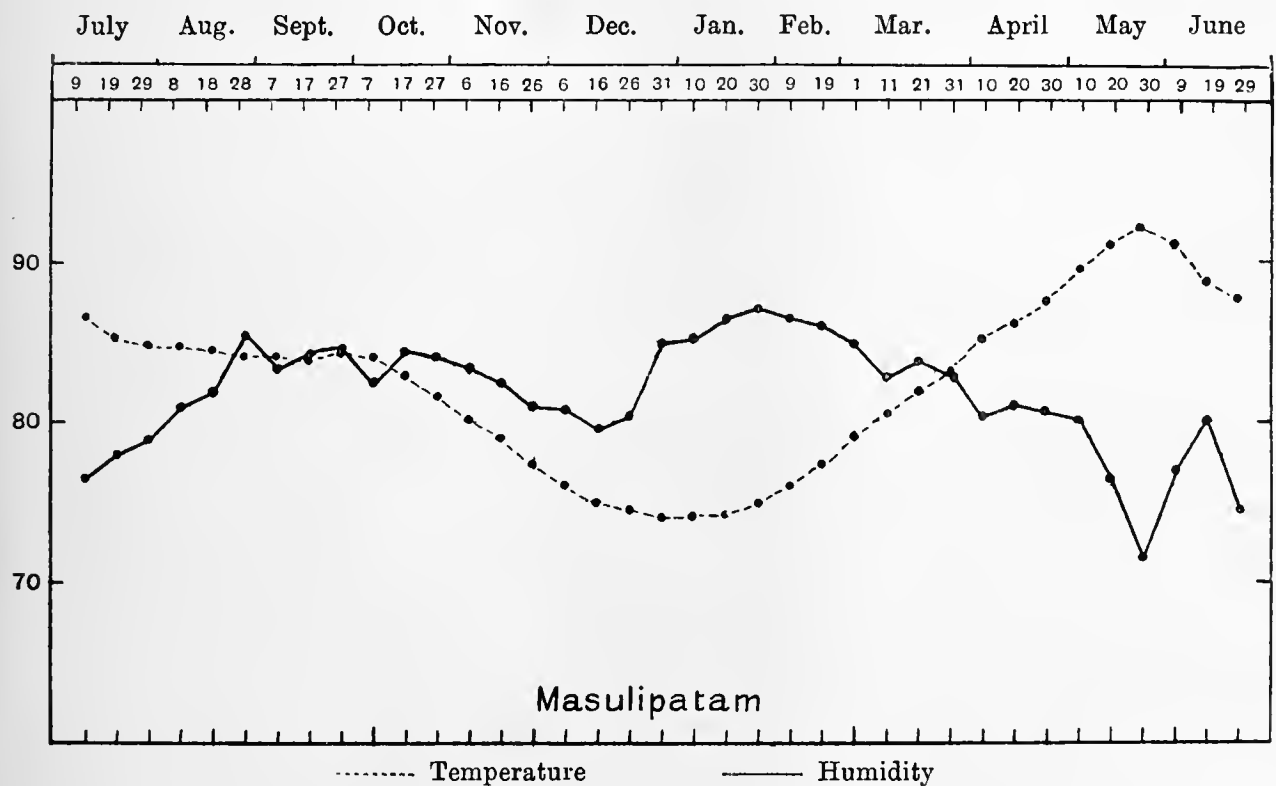


Chart 5

# 694 *Epidemiological Observations in Madras Presidency*

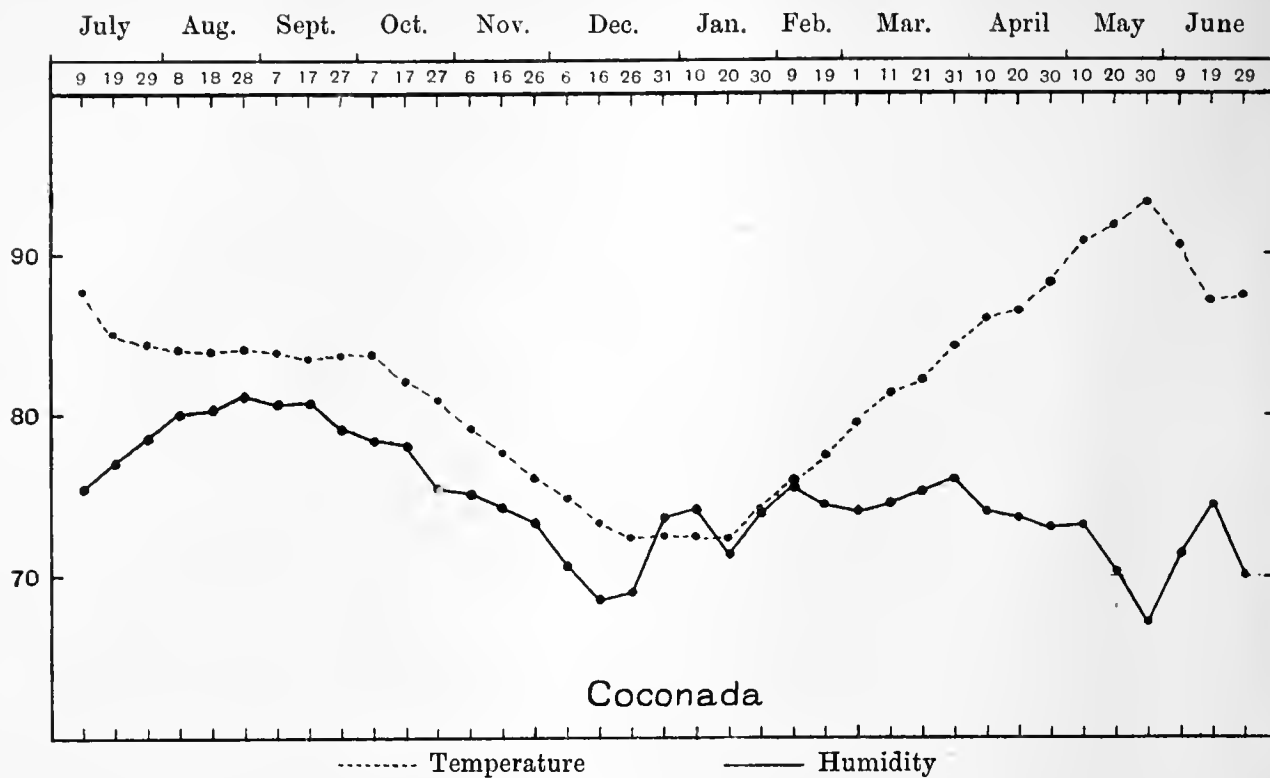


Chart 6

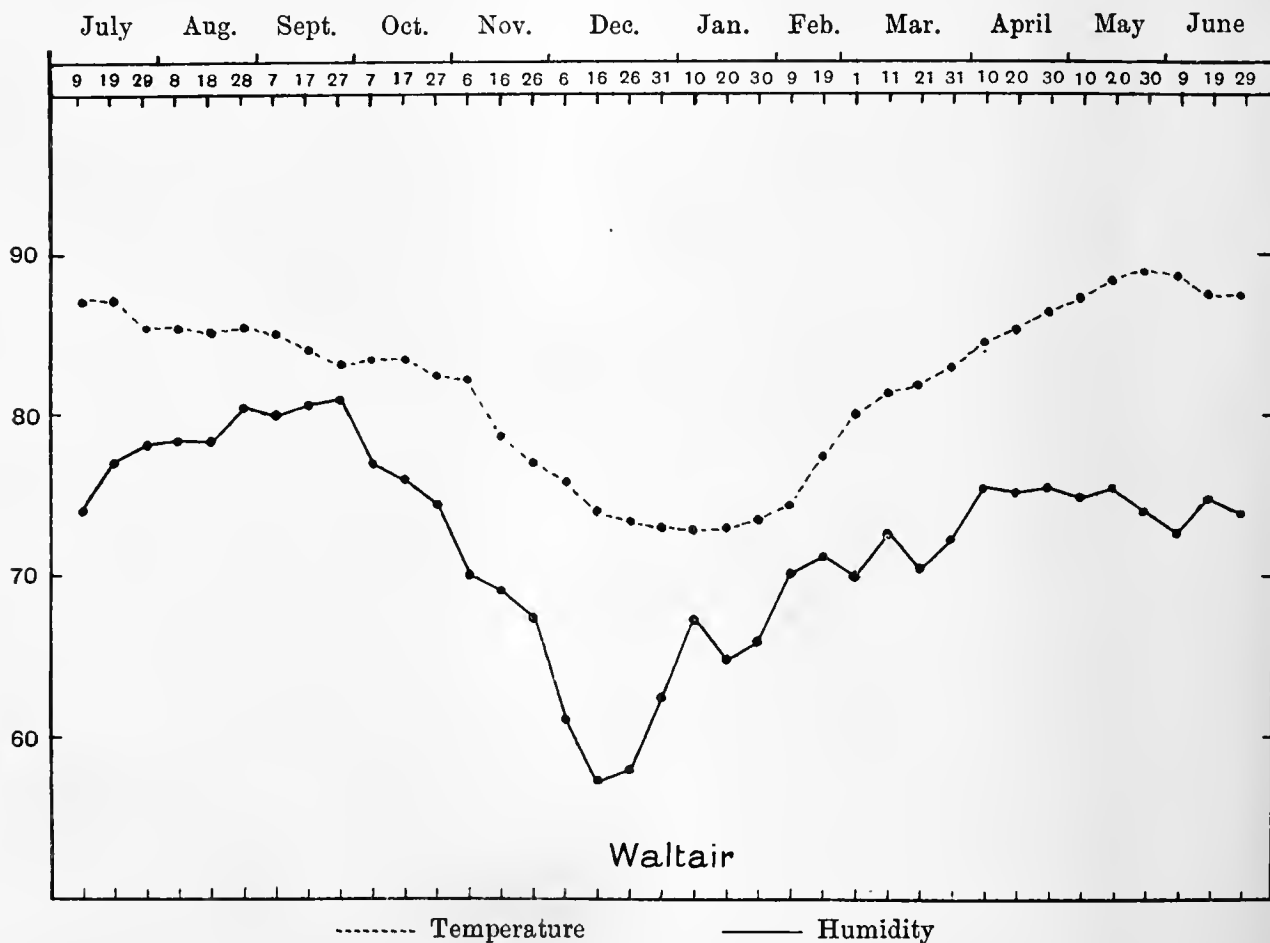
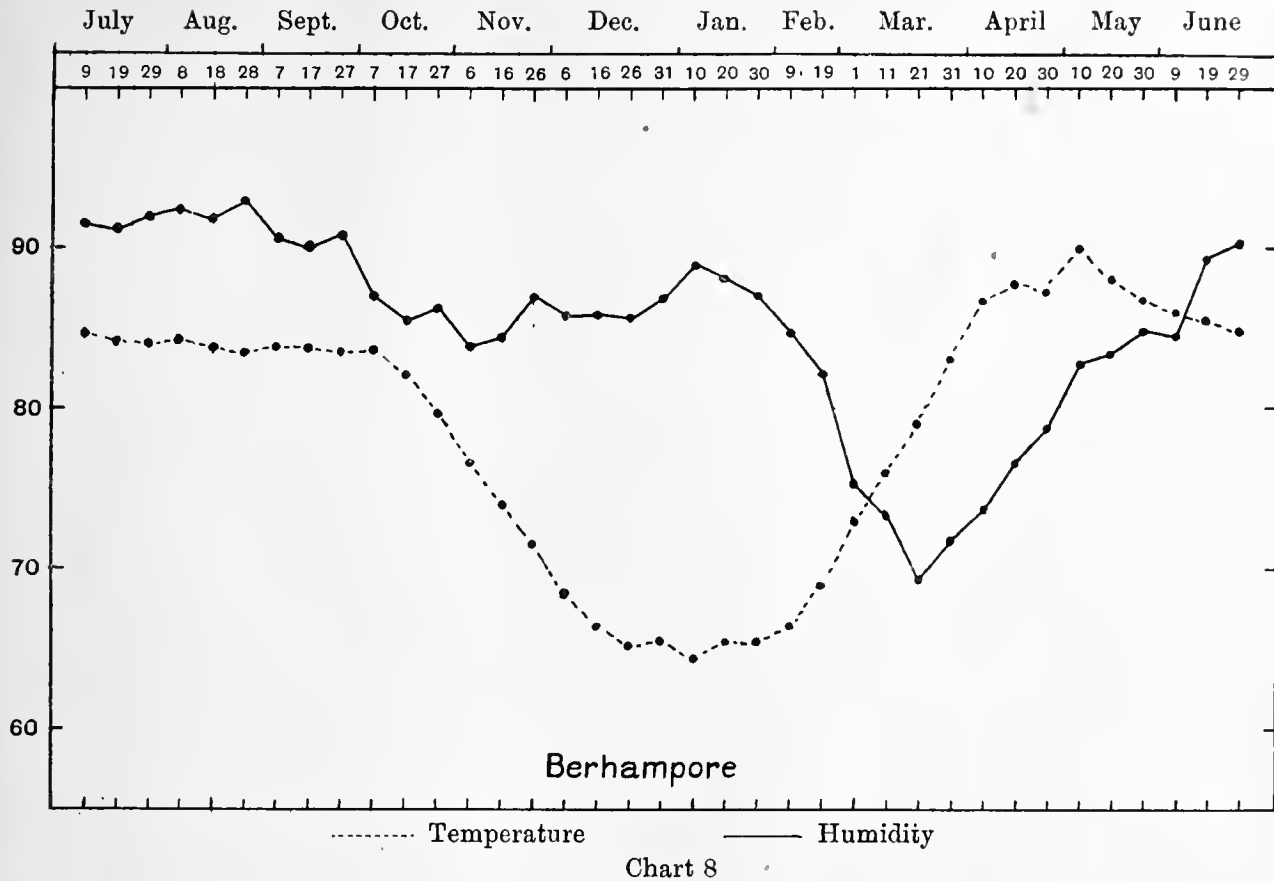


Chart 7

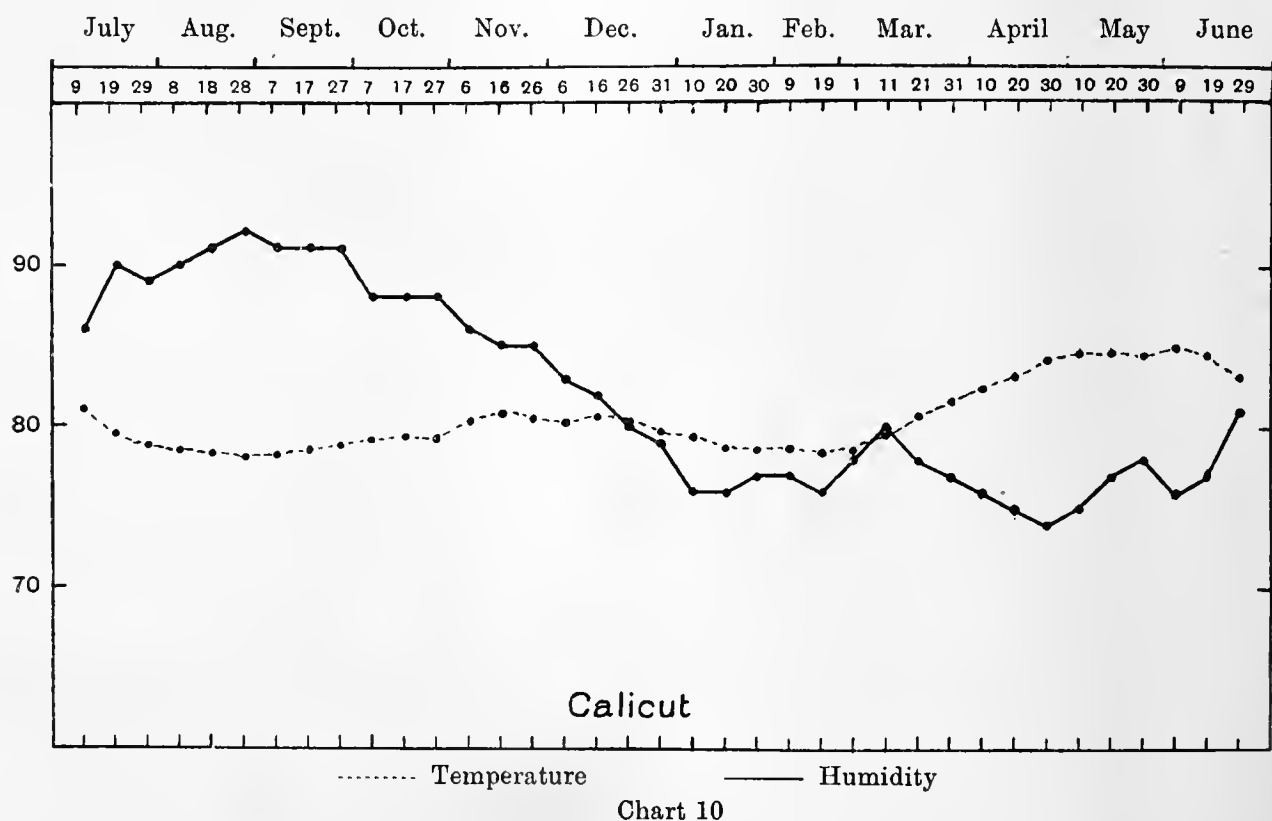
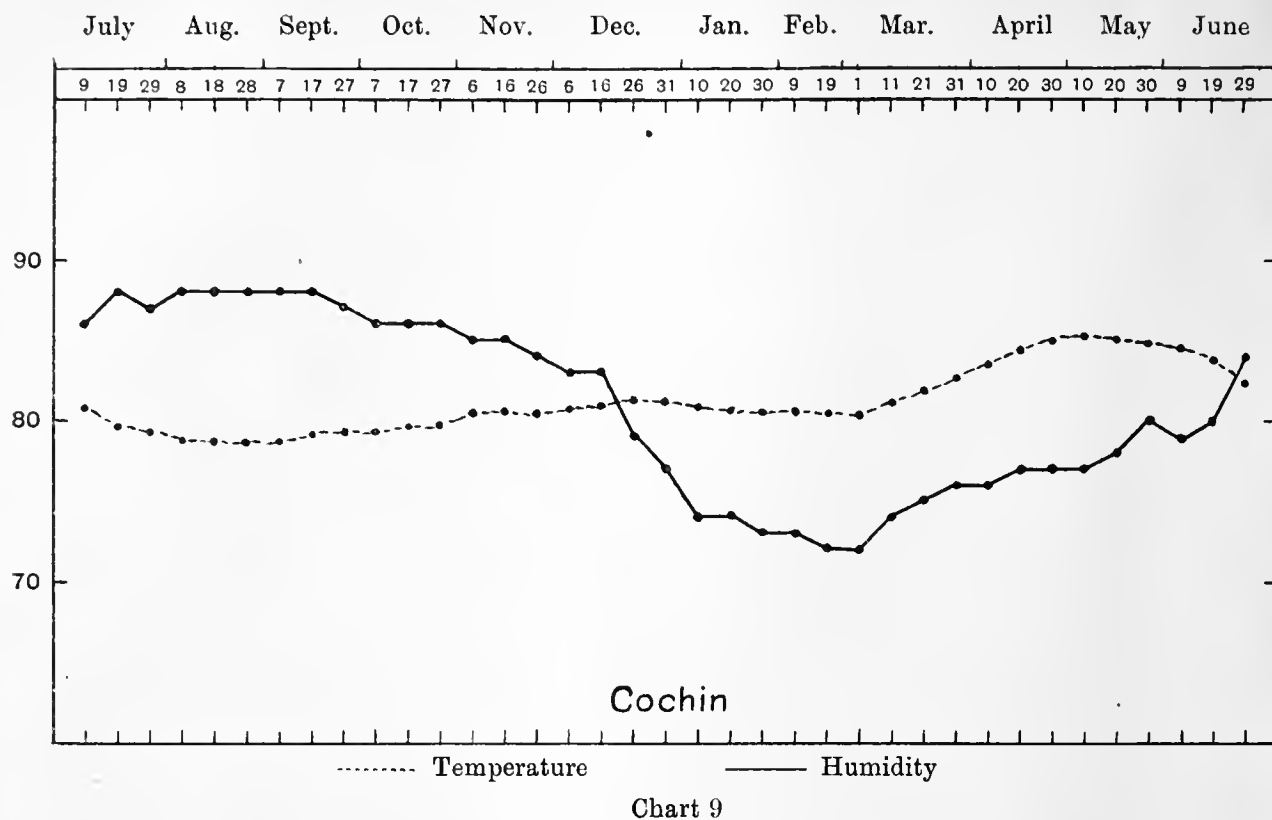


the places south of it, while the places north of it have a relatively dry weather except Berhampore, which place again has a comparatively high relative humidity of over 80 % during this season.

We may next examine the charts of the West Coast stations (Charts 9—11). Beginning with Cochin in the native state of the same name, in the south (Chart 9), we pass northwards to Calicut in Malabar (Chart 10) and to Mangalore in South Canara (Chart 11). The climate of these three places is very similar, the more northerly being slightly cooler than the more southerly; the mean daily temperature ranges closely around 80° F. for the greater part of the year and never falls below 78° F. The relative humidity is over 80 % for nearly half the year from June to December, *i.e.* the summer and autumn months, while for the rest of the year it is over 70 %.

If we now contrast the East Coast stations with the West Coast stations, the most striking feature is found in the fact that while the East Coast stations show a mean temperature well above 80° F. in the months of July, August and September, on the West Coast these are the coolest months of the year, the mean temperature being just below 80° F. On the other hand, the months of December, January and

# 696 *Epidemiological Observations in Madras Presidency*



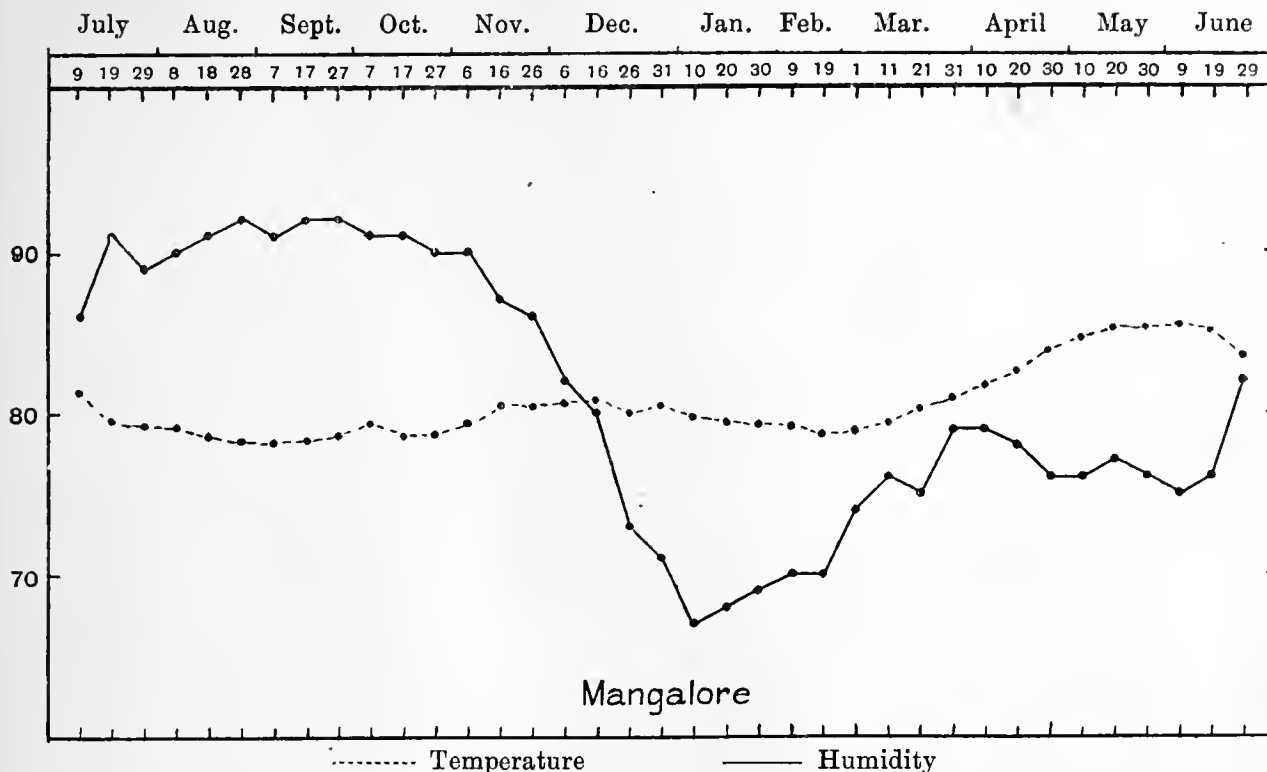


Chart 11

February are the cold weather months on the East Coast, the mean temperature during these months being well below 80° F. These too are the months of the greatest relative humidity on the East Coast contrasting in these respects with the West Coast stations, which are hotter and less humid in the winter. These striking differences in the climate of the East and West Coasts of the Madras Presidency are accounted for by the monsoon currents.

The main rain-bearing wind of India is the South-West monsoon which breaks on the southern parts of the West Coast of the Madras Presidency towards the end of May or beginning of June. It continues to advance for three months, and then recedes. The rainfall on the West Coast districts during the advance of the monsoon is very high—over 100 inches. The greatest precipitation takes place in the inland portions of the South Canara district on the slopes of the Western Ghauts and in the similar portion of Malabar. That portion of the advancing South-West monsoon which sweeps into Northern India from the Bay of Bengal causes some rainfall in the northern portion of the Madras Presidency, so that Berhampore, Waltair and Cocanada are more humid at this than at any other season of the year. The Western Ghauts cut off the greater part of the advancing South-West monsoon from the South-East Coast and the central portion of the Presidency;

this area, however, is supplied with rain as the monsoon retreats down the Bay of Bengal. The current in its retreat down the Bay during the months of October, November and December usually recurves near its northern limit of extension and is determined on the East Coast of the Madras Presidency. It increases in amount as it proceeds southwards and is greatest on the South-East Coast districts. Like the advancing monsoon current, that current of the retreating monsoon which falls upon the East Coast is cut off by the Western Ghats from the West Coast stations so that the rainfall during this period is most general just in those parts of the Presidency where the rainfall of the advancing South-West monsoon was most scanty and irregular in distribution. A comparison of the average rainfall for 25 years in Madras City with that in Calicut shows that Madras receives sixteen inches in June, July, August and September and thirty-one inches in October, November and December, while Calicut receives eighty-seven inches in the first period, and sixteen inches in the second, of which quantity ten inches fall in October.

The effect of the advance of the South-West monsoon is thus seen in the relatively low temperature and high humidity of the West Coast stations during the months June to October, while the effect of the retreating monsoon is seen in the relatively low temperature and high humidity on the East Coast stations, especially in the south, during the months November to February.

Passing next to the inland observatories situated on the low-lying plains (Charts 12—15), we may examine the charts first of Tinnevely in the extreme south of the Peninsula (Chart 12) and, passing northwards, those of Madura (Chart 13), Trichinopoly (Chart 14), and Cuddapah (Chart 15), each situated in the districts of the same name. In Tinnevely the mean daily temperature is below 80° F. for only two months in the year and even then never falls below 79° F. As we pass from station to station in a northerly direction the period during which the temperature is below 80° F. extends slightly and the mean temperature falls gradually, but the period during which the temperature is below 80° F. never exceeds three months, nor does the temperature fall below 75° F. even in the most northerly station. All the stations too, although presenting a maximum relative humidity during the cold season, are nevertheless comparatively dry, the relative humidity seldom exceeding 80 % although during the whole of the cold season it is above 70 %. These stations are on the whole hot and dry.



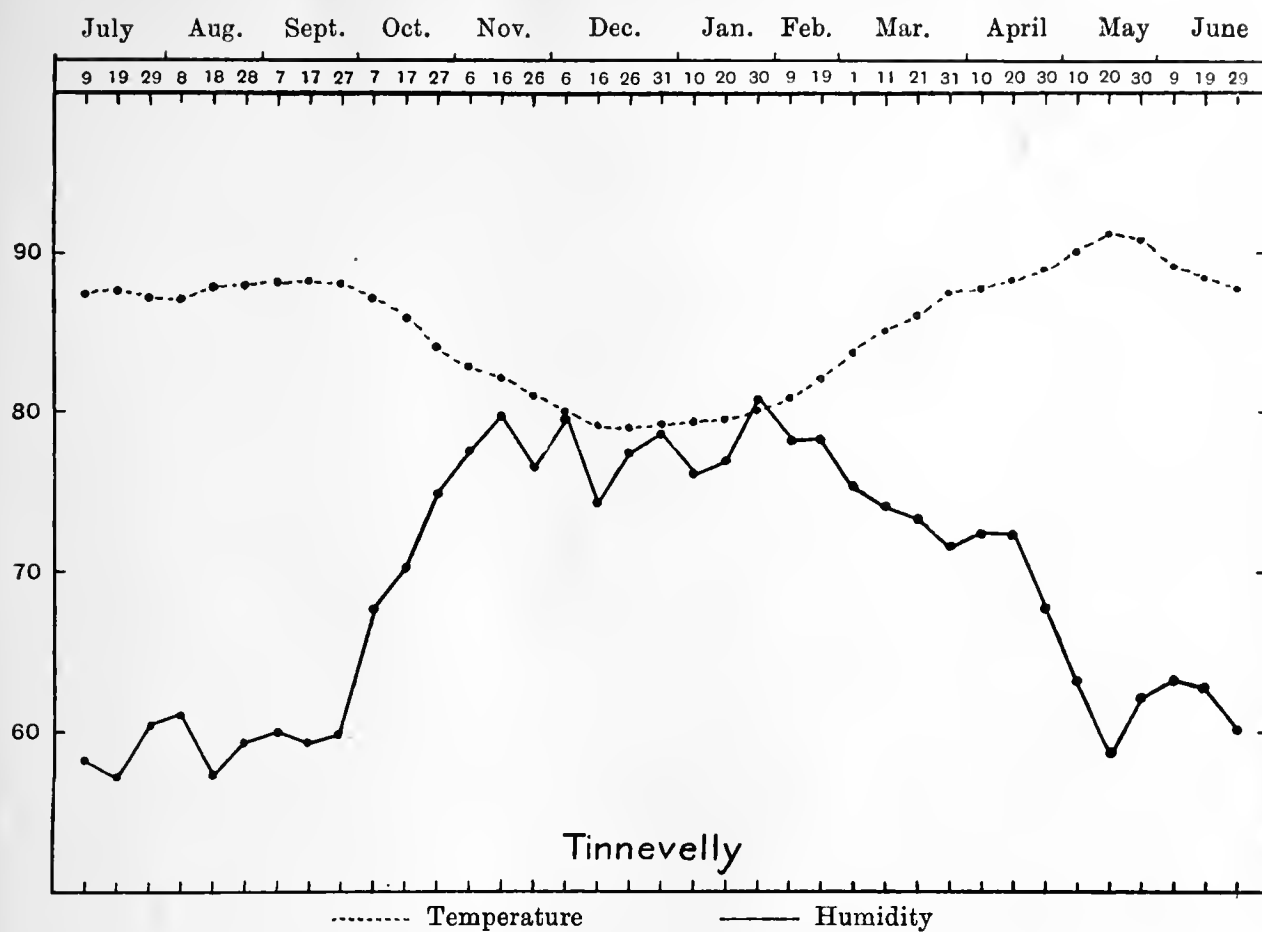


Chart 12

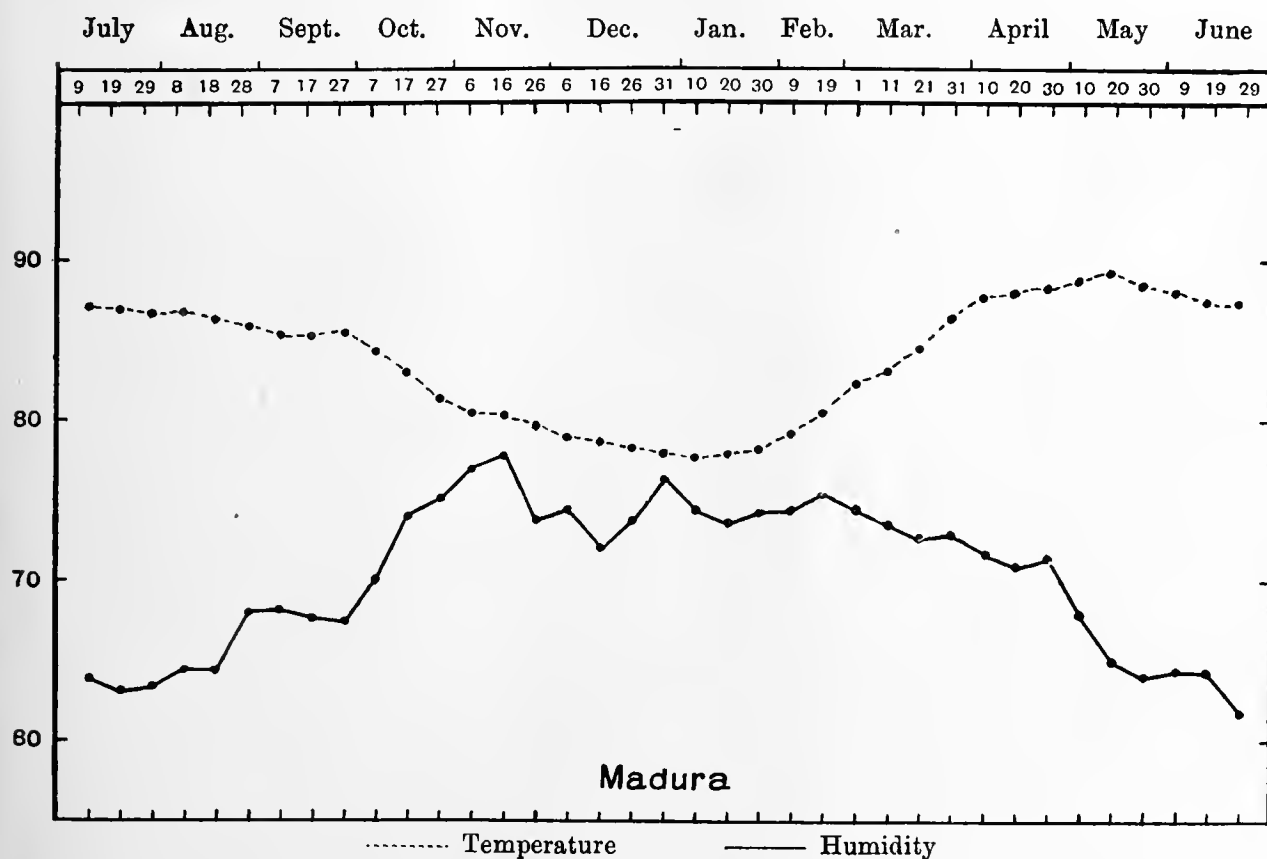
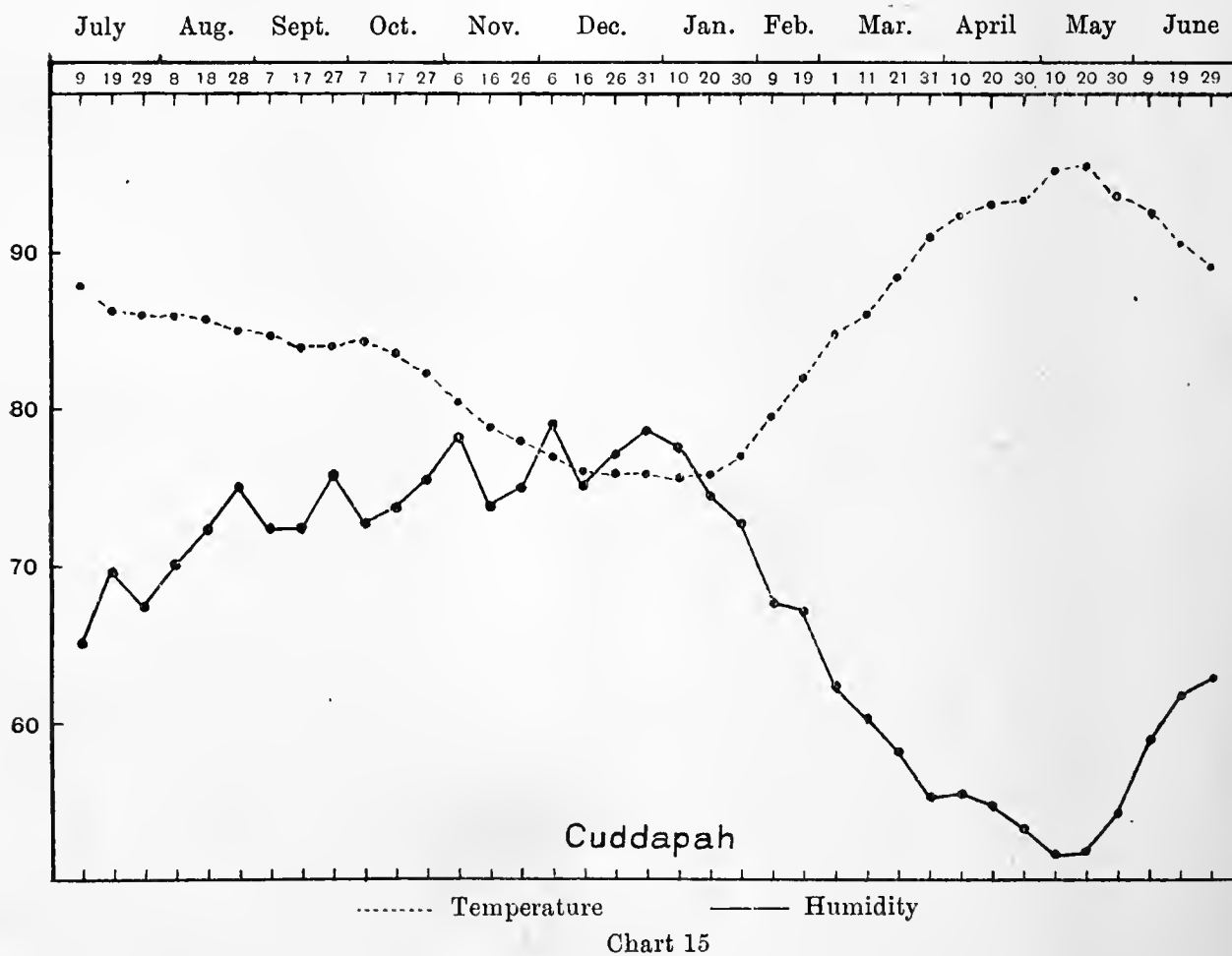
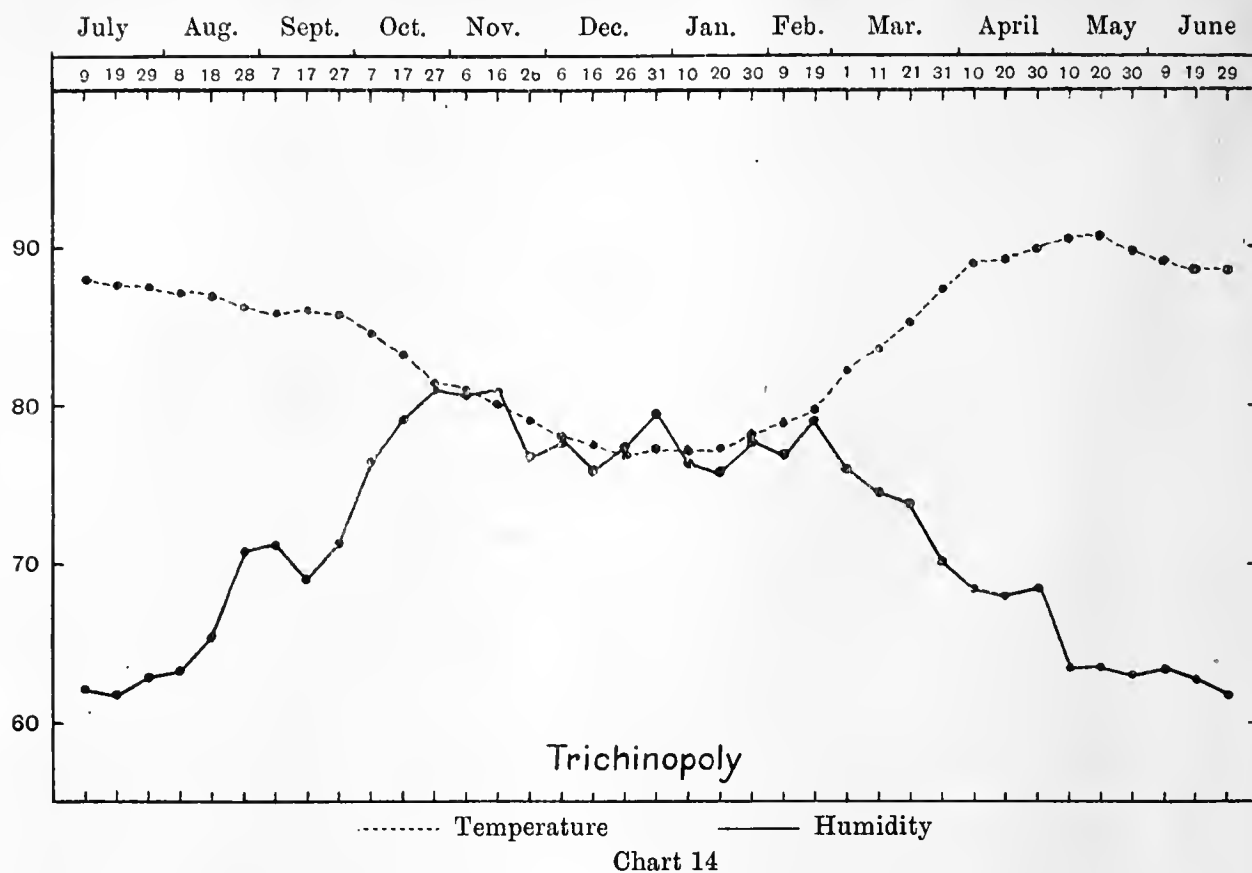


Chart 13

700 *Epidemiological Observations in Madras Presidency*



Passing to certain more elevated observatories situated at or about 1000 feet above the sea (Charts 16—19), we may consider the charts of Salem 940 feet (Chart 16), Kurnool 924 feet (Chart 17), Bellary 1475 feet (Chart 18), and Coimbatore 1348 feet (Chart 19) above the sea. Each of these places is situated in a district of the same name. The charts of Salem and Coimbatore resemble one another except that Coimbatore is distinctly more humid and slightly cooler than Salem. The charts of Bellary and Kurnool closely resemble one another, Bellary being if anything slightly hotter and drier than Kurnool. The feature of these charts is that, except for Waltair and Berhampore, Bellary and Kurnool are cooler in the winter than any other places we have examined in the Presidency; at the same time they are very dry especially Bellary, where the relative humidity seldom rises above 70%. Coimbatore, situated opposite the Palghat Gap in the Western Ghats, receives the cool South-West monsoon wind, so that from June till March the mean daily temperature is about or below 80° F.

There remains to be considered the climatic conditions of the Mysore Plateau and of the Nilgiri Hills. Bangalore (Chart 20) has been selected as representing the climate of the Mysore Plateau; this observatory is situated 3021 feet above the sea. Chart No. 20 shows that, except for a very short period of the year during March and April, the mean daily temperature is below 80° F. and, during the cold weather months December and January, is below 70° F. The relative humidity too for the greater part of the year is above 70% and from June to September it is above 80%. So far as our experience of plague in other parts of India is a guide, the climate of Bangalore is eminently favourable for plague epidemics and we find as a matter of fact that plague has been very severe here.

Wellington observatory is situated in the Nilgiri Hills at an elevation of 6200 feet above the sea (Chart 21). Here the mean daily temperature seldom rises above 67° F. and falls as low as 56° F. for more than a month and a half. The climate thus resembles that of places situated in the temperate zone rather than of those situated in the tropics.

We can now contrast the charts which have been prepared for stations in Madras with those of certain other selected plague-infected places in India (Charts 22—26), namely Belgaum, Poona, Lucknow, Lahore, and Rawalpindi. Taking first Belgaum the chart resembles that of Bangalore except that Belgaum (Chart 22) is cooler

702 *Epidemiological Observations in Madras Presidency*

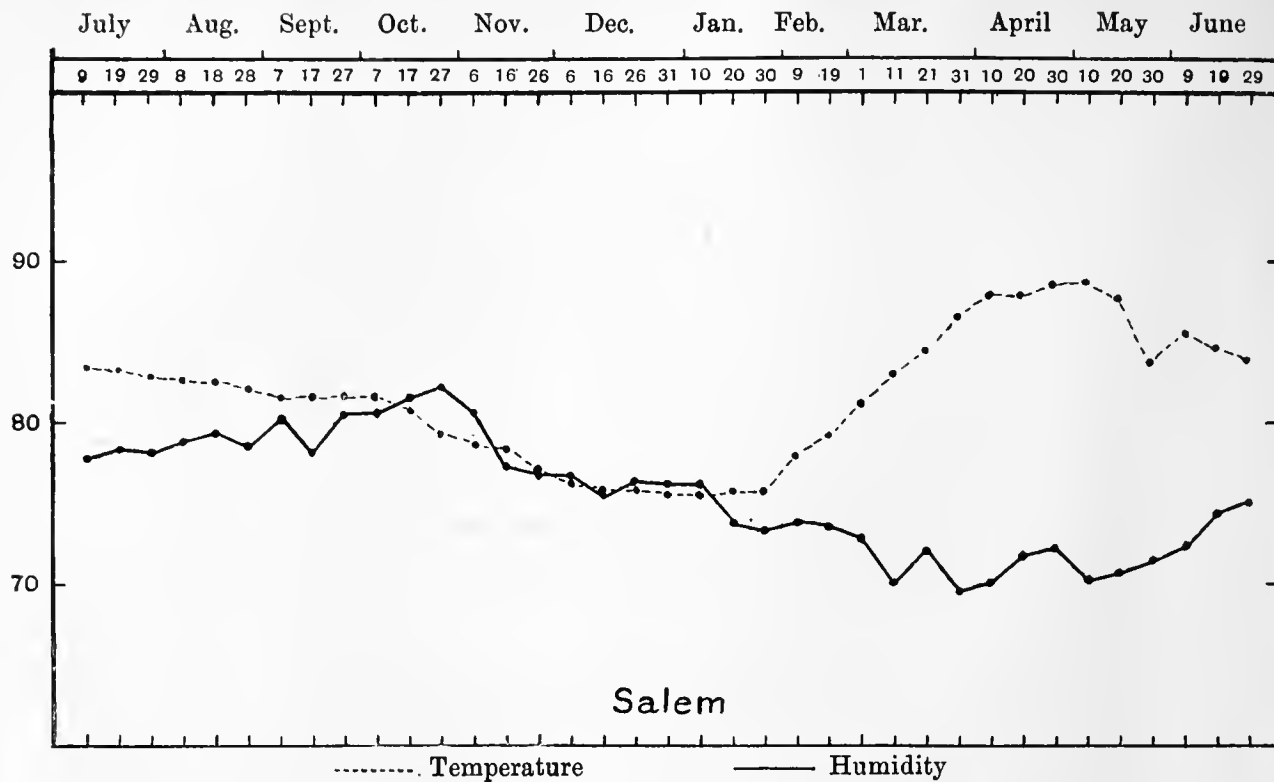


Chart 16

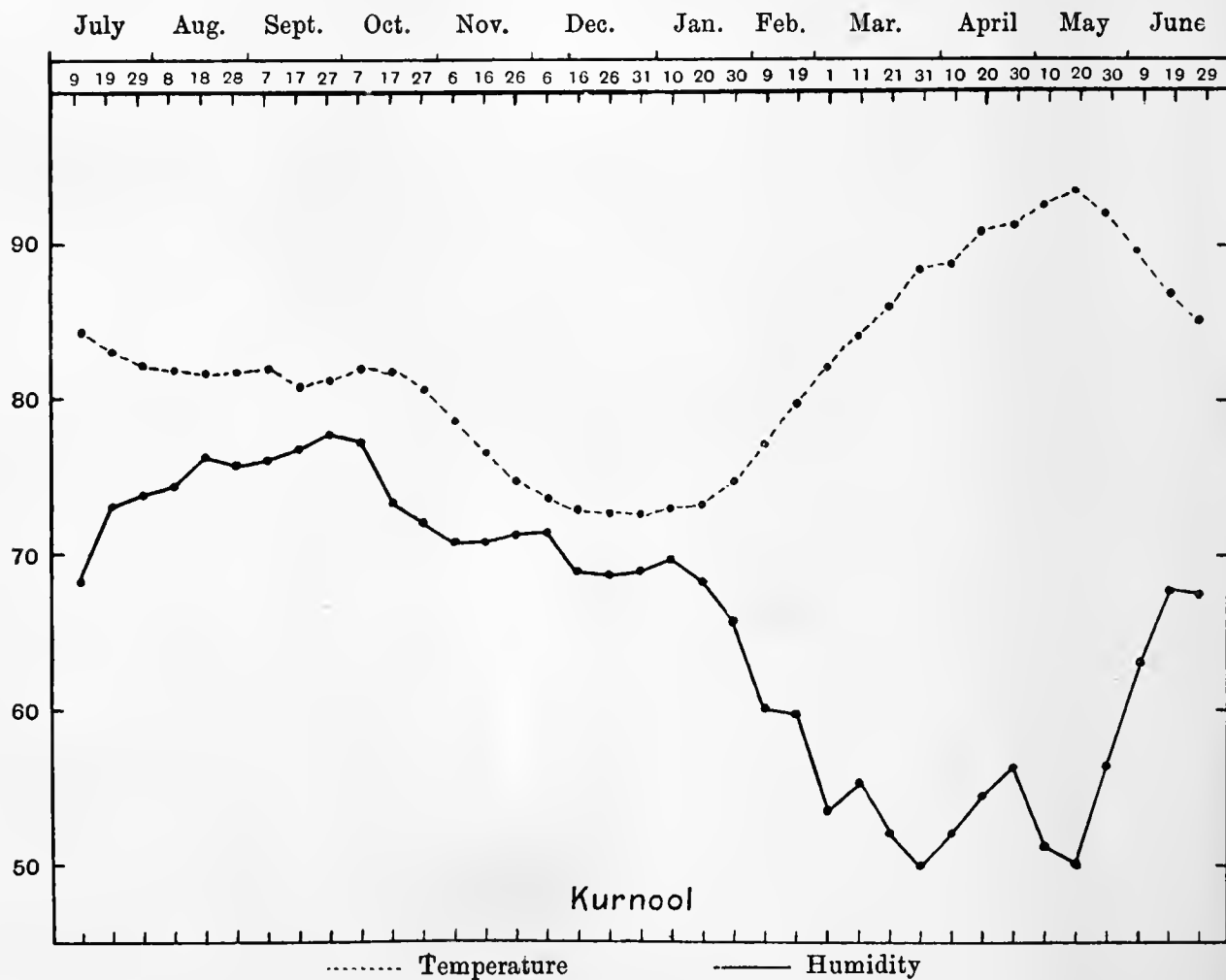
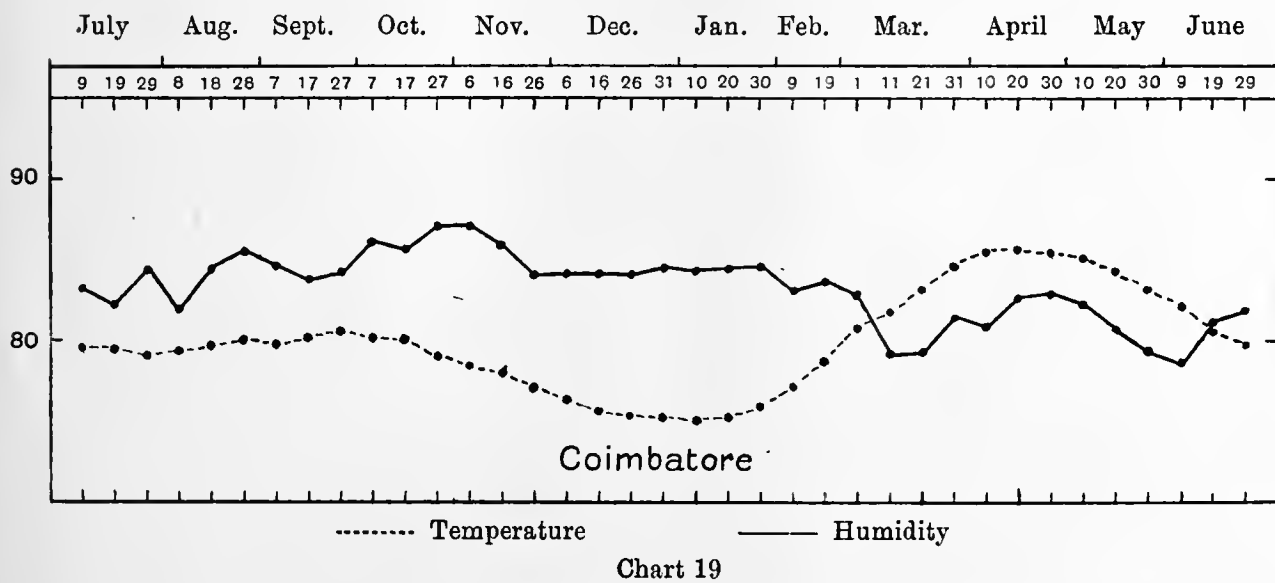
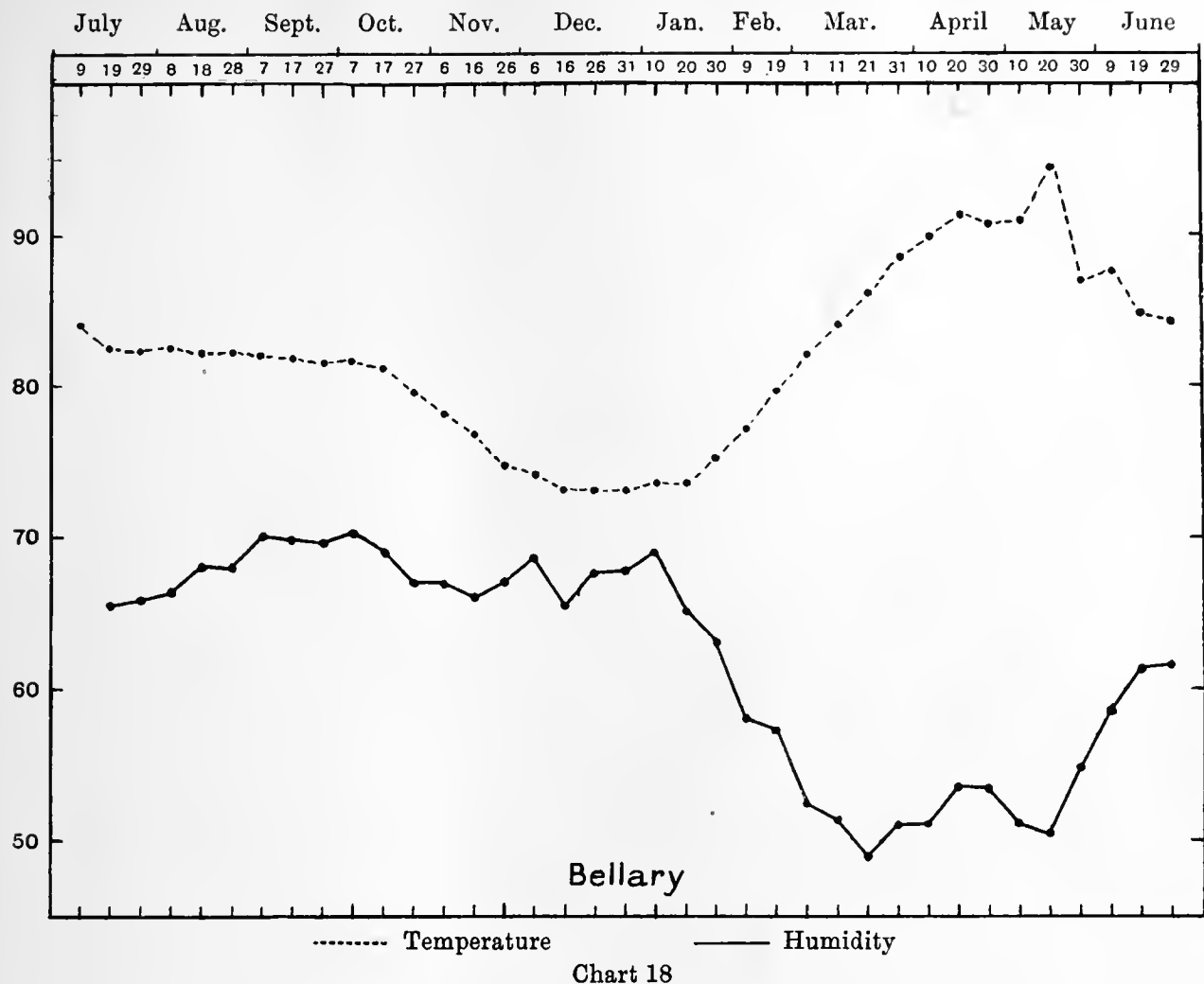
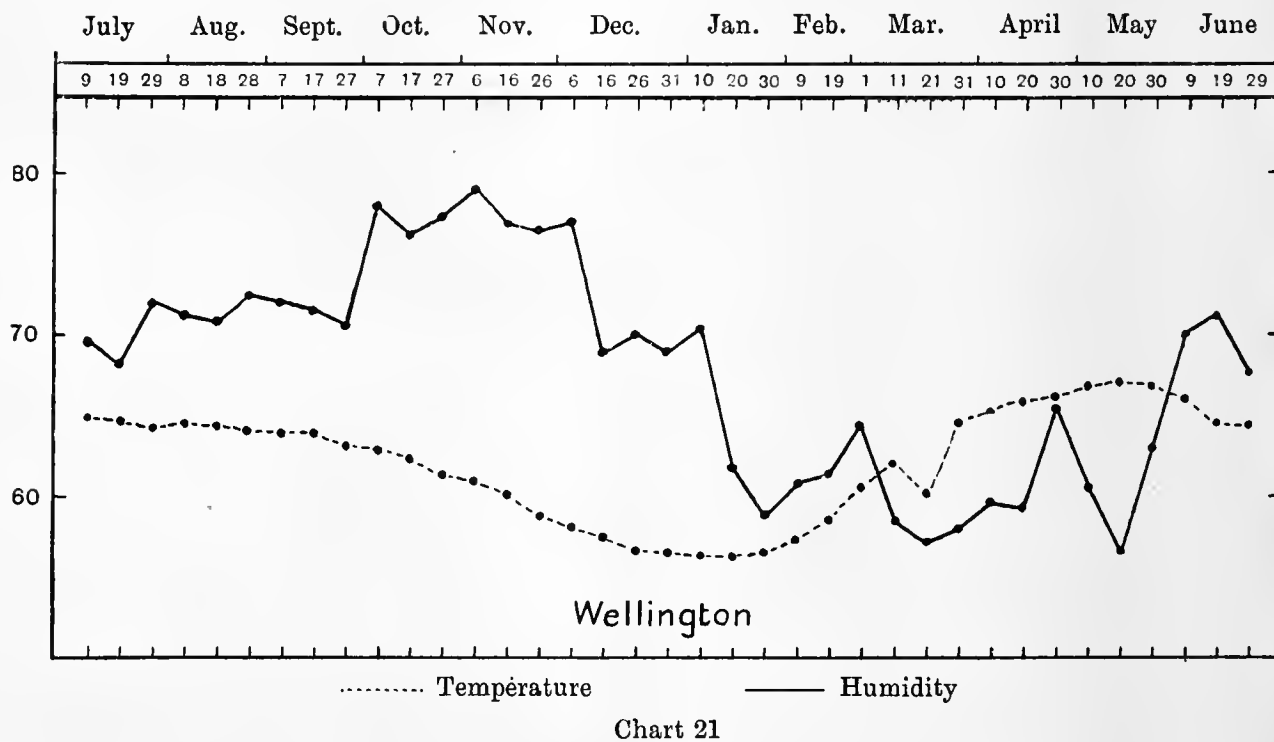
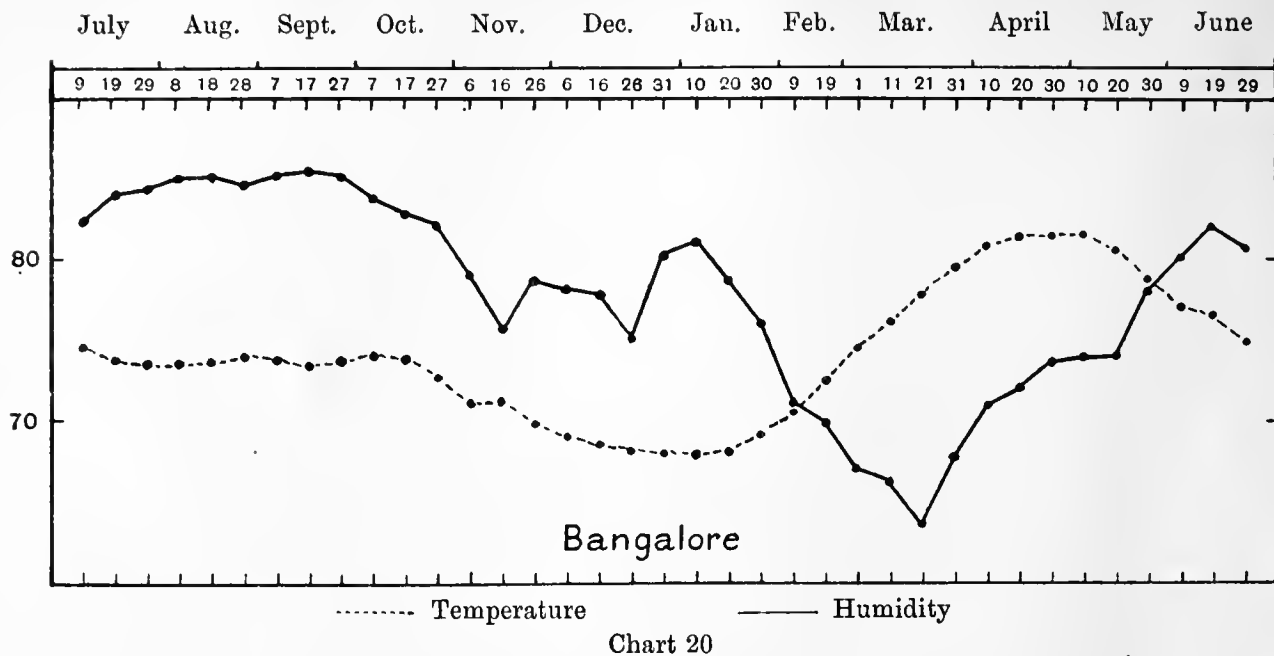


Chart 17



# 704 *Epidemiological Observations in Madras Presidency*



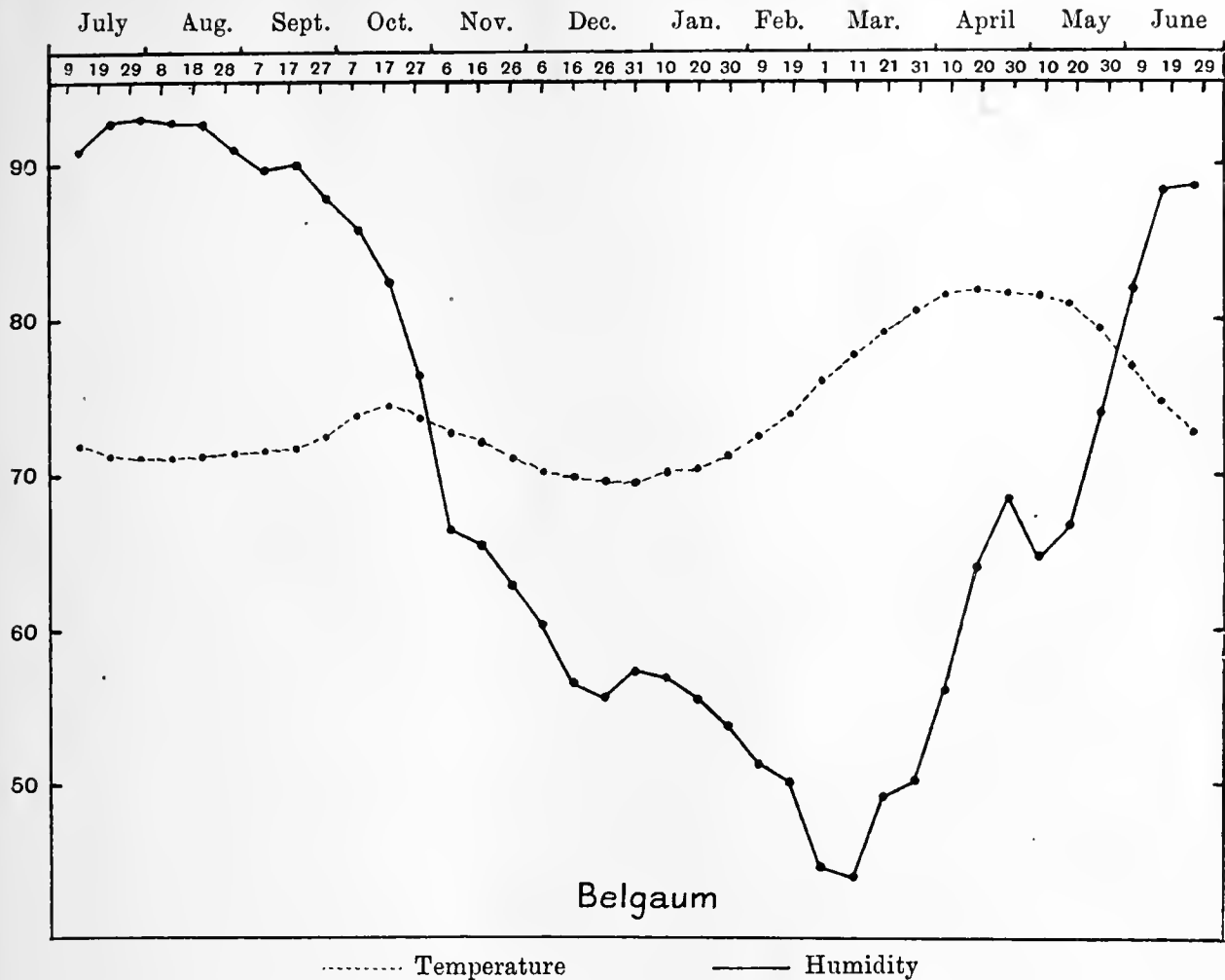


Chart 22

and more humid in the summer months but drier during the rest of the year than Bangalore. The climate of Poona (Chart 23) closely resembles that of Belgaum but is on the whole hotter and less humid. The charts of Lucknow (Chart 24), Lahore (Chart 25) and Rawalpindi (Chart 26) resemble one another except that, as was to be expected, the more southerly of these places have a slightly warmer winter than the more northerly; the mean daily temperature in all these towns, however, is very considerably lower in winter than that of any place in Madras except in Wellington. In Lucknow the mean daily temperature falls to 60° F. and in Rawalpindi as low as 50° F. At this season the temperature is well suited for the prolonged existence of the rat flea when separated from its host, a condition favourable for the dissemination of plague infection. The climate of these severely plague-infected towns thus contrasts markedly with the

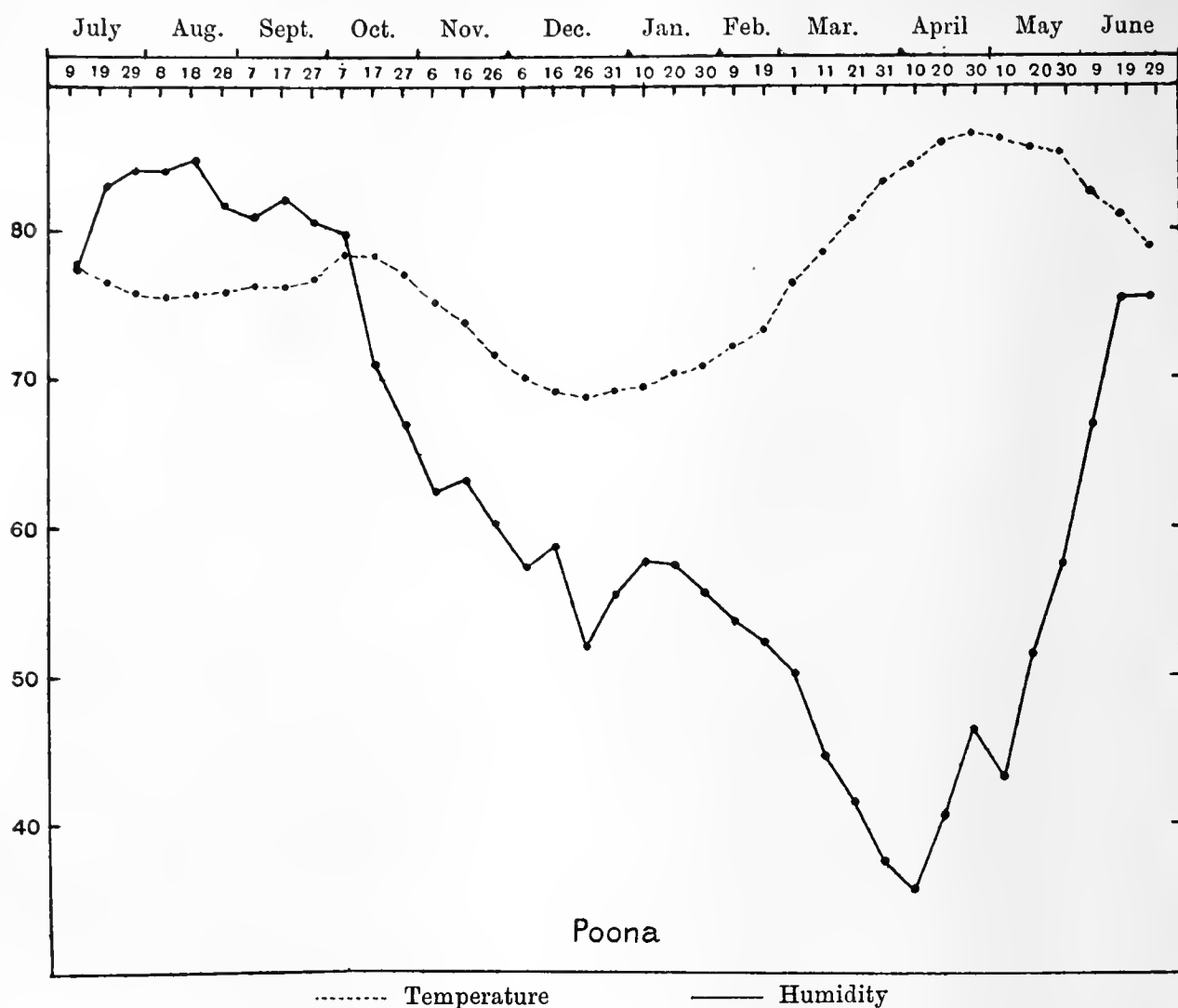


Chart 23

warmer climate met with in many of the places in the Madras Presidency. We must draw attention, however, to the fact that the climate of Rangoon where plague has to some slight extent been prevalent, differs but little from that of Madras City and the towns on the East Coast of the Presidency. In the case of Bombay City also, where plague first entered India and where the disease has prevailed without interruption since 1896 to the present date, though the climate is distinctly cooler in the early months of the year, especially during February, March and April, the plague season of that city, it differs but little from Madras City which is often cooler during the months of November and December. These facts are shown in the following statement.



# *Reports on Plague Investigations in India* 707

*Table showing the normal monthly mean between maximum and minimum temperature and the normal monthly mean of the humidity at 8 hours in Bombay, Madras and Rangoon.*

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<b>BOMBAY</b>												
Temperature	75.2	75.7	79.5	83.2	85.7	83.5	81	80.5	80.5	82	80.1	77.1
Humidity	72	71	75	75	73	83	86	86	87	82	74	72
<b>MADRAS</b>												
Temperature	76.1	77.4	81.1	85.1	89.8	89.7	87.3	85.6	85	82.1	78.8	76.6
Humidity	82	82	78	74	64	63	69	75	78	83	83	83
<b>RANGOON</b>												
Temperature	76.6	79.4	83.8	87.4	84.6	81.5	80.6	80.4	80.8	81.7	80	77.3
Humidity	83	85	85	80	88	93	94	94	94	92	87	83

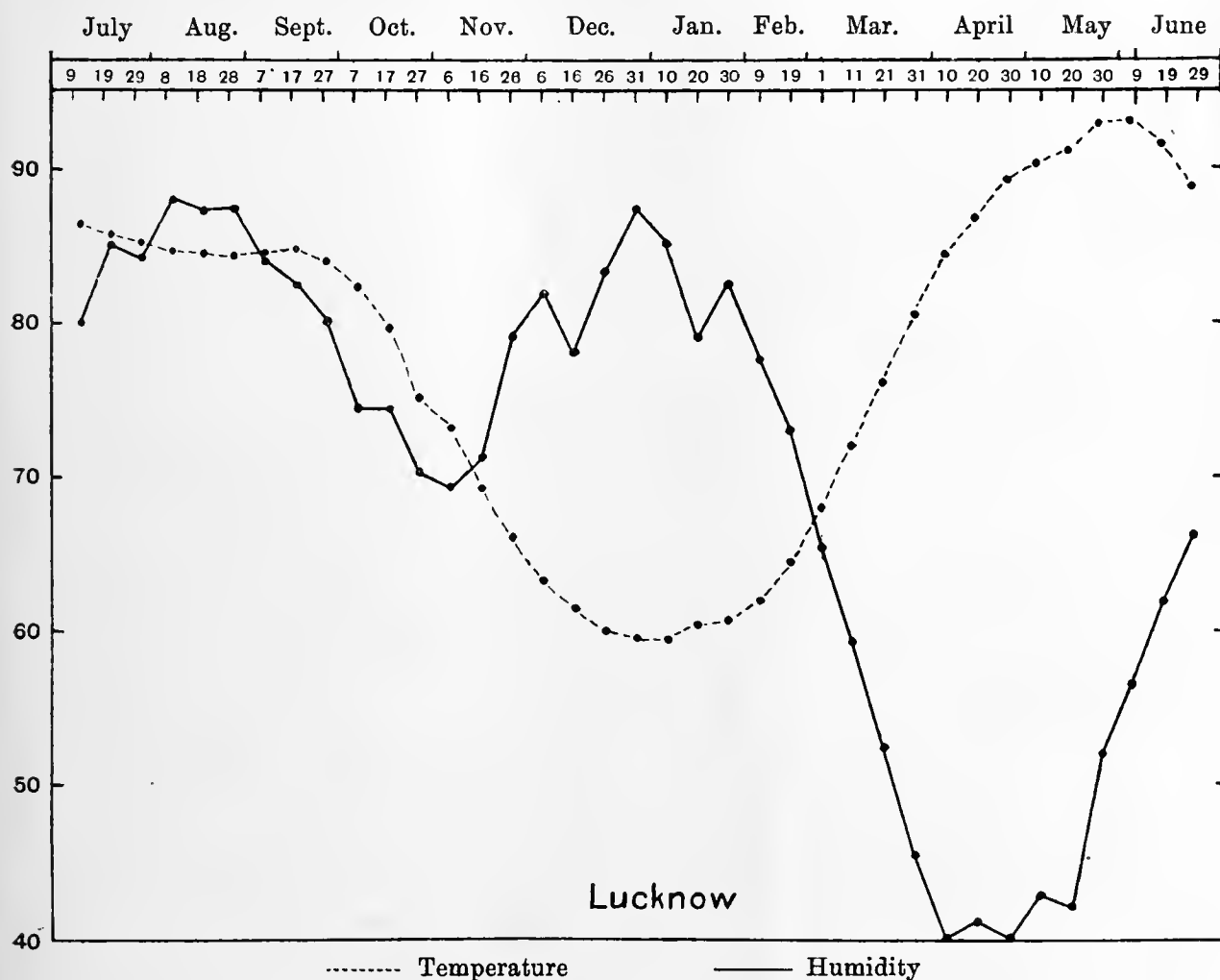


Chart 24

IV. *The distribution of plague in the Madras Presidency.*

The facts which we have at our disposal for examining the distribution of plague in Madras Presidency are those which are published in the Annual Reports of the Sanitary Commissioner with the Government of Madras. The figures which are used by this officer in his reports are compiled from municipal and village reports which in turn are prepared, for the most part, by men who have very little knowledge of medicine, although their work, so far as is possible, is examined and verified by the medical and civil officers of Government. Owing to the nature of the agency employed in collecting these statistics, they cannot be regarded as absolutely accurate especially in regard to the diagnosis of the causes of sickness and death. It has been observed that under certain circumstances where perhaps a specially keen outlook has been kept for the appearance of plague, as for instance when a place is in imminent danger of becoming

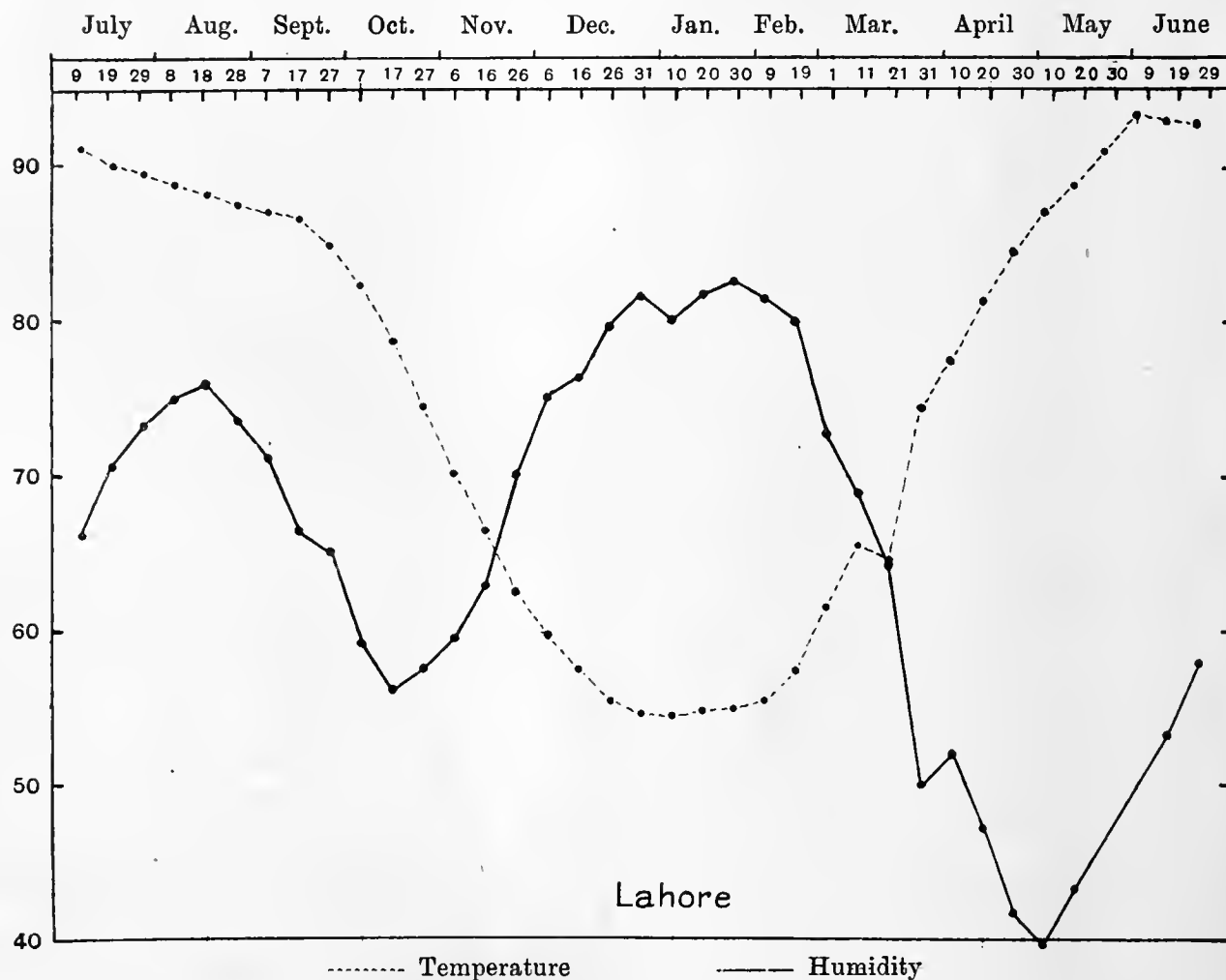


Chart 25

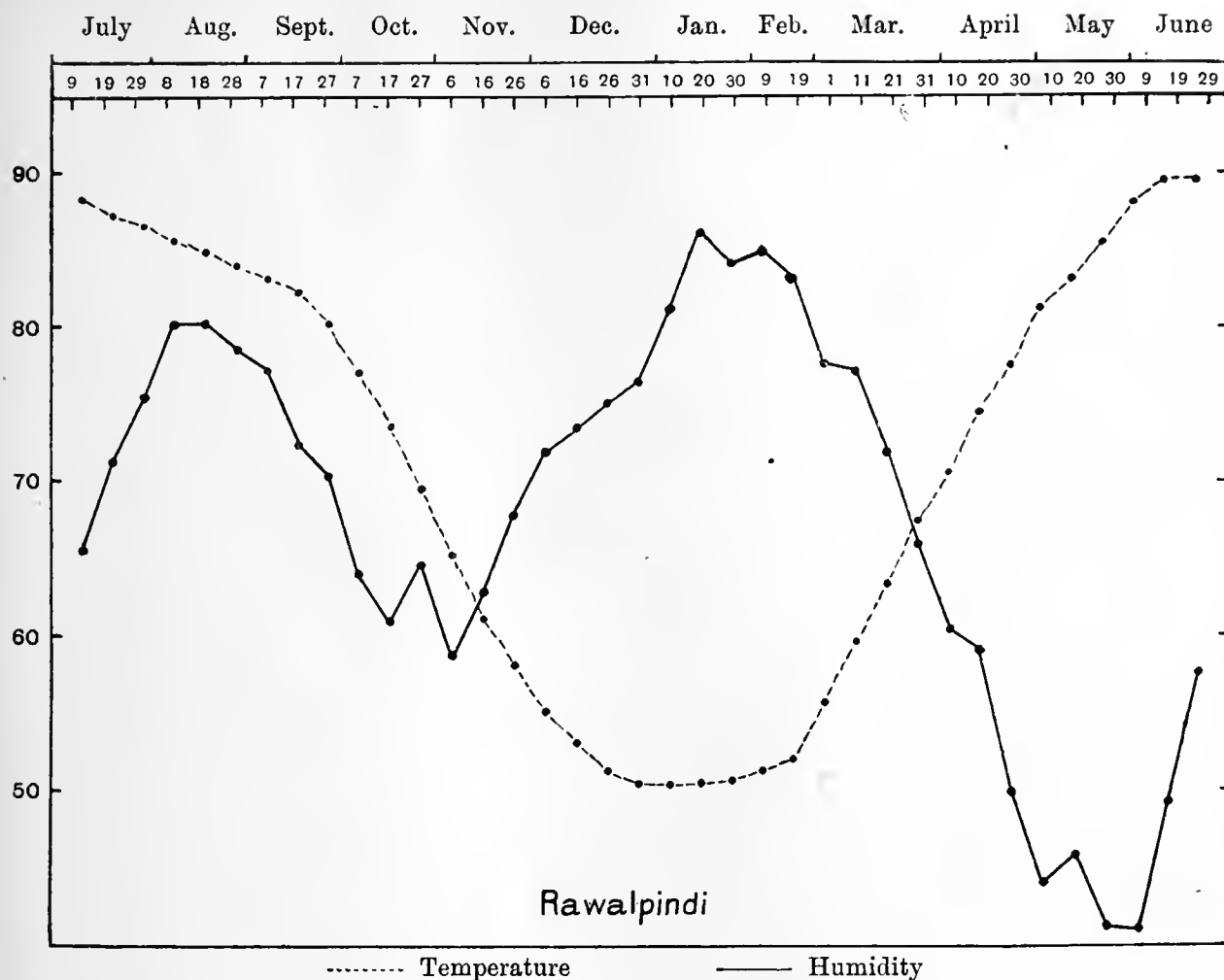


Chart 26

infected, persons suffering from other diseases than plague have been reported to have been attacked by that disease. Again cases of plague have been overlooked in places where for one reason or another the advent of such cases was not suspected. Such errors are particularly liable to occur either before plague is established in a place, that is, before the authorities are aware of the presence of the disease, or when a person who is supposed to be attacked by plague recovers from his illness.

The figures published by the Sanitary Commissioner are, therefore, likely to be liable to error: first, in respect of the number of attacks as opposed to deaths from the disease and secondly, in respect to the number of imported cases. For these reasons, in considering the distribution of plague in Madras Presidency, we have used figures which refer to reported deaths from plague, leaving out of consideration the number of persons reported to be attacked by the disease. We have also disregarded reported deaths from plague when, in any single

## 710 *Epidemiological Observations in Madras Presidency*

year, these deaths have not exceeded 10 in any single district; if more than 10 deaths from plague have been reported in any year in a district we have assumed, generally correctly, that the disease was present in the form of an epidemic in some part or parts of the district, that the disease was in fact indigenous in the district. It must be noted however that the figures given for the years in which plague was indigenous in the districts include the deaths of some persons who were not infected in the district but derived their infection in some other district; the error from this cause is, however, generally very small.

In considering the distribution of plague in Madras Presidency, we may state, at the outset, that cases of plague are reported to have been introduced into every district of the Presidency. The smallest number of imported cases have been noted in the Vizagapatam, Godavari, Kistna, Guntur and Tinnevely, the most remote districts from plague-infected centres, and these districts with Ganjam, Nellore and Tanjore have been entirely free from epidemics of plague. Limited epidemics have broken out in the following districts: Trichinopoly 25 deaths; South Arcot 37 deaths; Chingleput, a few pneumonic cases; Madras 78 deaths; Cuddapah 283 deaths; Madura 847 deaths. The remaining districts in the Presidency have been more or less severely affected; they were attacked by the disease with other districts mentioned above in the following chronological order:

District	First infected	Total plague deaths in the year in which plague was epidemic in the district
Bellary	1898	31,510
Salem	1898	13,312
North Arcot	1898	3,550
Anantapur	1898	3,507
Kurnool	1898	1,042
Coimbatore	1899	12,006
South Canara	1902	3,010
Nilgiris	1903	1,403
Cuddapah	1904	283
Madura	1904	847
Madras City	1905	78
South Arcot	1906	37
Malabar	1906	1,026
Trichinopoly	1910	25

It should be noted that plague first appeared in the Madras Presidency in the latter part of the year 1898. The Mysore State was first affected in August of the same year, while the disease was discovered in Bombay towards the close of the year 1896. From the

outbreak of plague in the Madras Presidency in 1898 up to the end of the year 1910, a period of 13 years, this disease has been responsible for 72,000 deaths in that area. This is an insignificant number of deaths when compared with the population of the Presidency and gives a death rate from this cause much smaller than in many other parts of India, or, in the case of the Presidency itself, from other epidemic diseases, such as cholera and smallpox.

From the statement given below it will be seen that the districts which were infected in the earliest years of plague in the Presidency have returned the great majority of deaths. Thus the districts infected in 1898 and 1899 return 64,987 deaths, or 90·6% of the total, and, if the districts infected before 1904 be taken, these show a plague mortality of 69,340 during the years plague was indigenous in the districts, or 96·7% of the total.

The districts which were infected after 1903, Cuddapah, Madura, Madras City, South Arcot, Malabar and Trichinopoly return a total of only 2296 deaths during the years plague was epidemic in these districts, so that beyond the limits of the districts infected in the earlier years, plague has made extremely little progress.

In considering the distribution of plague in the Madras Presidency in greater detail, it will be convenient in the first instance to dispose of the districts which have been infected after 1903. The epidemics in Trichinopoly (25 deaths in 1910) and in South Arcot (37 deaths in 1906) may be dismissed with the statement that they were very limited and speedily terminated. The epidemic in Madras City in 1905 and 1906 has been fully discussed in a previous paper (*Journal of Hygiene, Plague Supplement*, 1913, pp. 209—212). This epidemic in Madras City, though mild, was protracted, the outbreak was confined to a few small hamlets on the outskirts of the town. The fact that the deaths have been few cannot under the circumstances of the outbreak be taken as evidence that Madras City is naturally a very unsuitable place for epidemic plague. It should be noted, however, that though more than 100 cases of plague are known to have entered the city, no epidemic developed in connection with them, and the origin of the single epidemic which occurred in 1905 and 1906, while it could not be definitely determined, was not connected, so far as the most searching inquiries could elicit, with any imported case of the disease.

Plague first became indigenous in Malabar in 1906 and since that date up to the end of the year 1910 some 1026 deaths have occurred, chiefly in the town of Calicut.

## 712 *Epidemiological Observations in Madras Presidency*

Plague in Madura district first appeared in epidemic form only in 1910, save for a very limited epidemic in the town of Palni in 1904. The disease has been mainly confined to the taluqs of Palni and Dindigul which on account of their elevation and proximity to the Palghat Gap enjoy a cooler climate than the other parts of the district. Up to the end of 1910 there had been 875 deaths from plague in the whole of the district; during the following year a few more deaths were recorded, raising the total to 1019. During the year 1912 the district was free from plague.

Plague in the Cuddapah district has occurred for the most parts in its southern portion in the taluqs of Madnapalli, Voyalpad and Kadiri. Here the district slopes gradually upwards from the hot Cuddapah valley to the cool Mysore plateau, and the climate, with increasing elevation, gradually approximates to that of the plateau, which we have pointed out, is eminently suited for the propagation of plague epidemics. Of the 283 deaths from plague which have occurred in this district, 172 fell in the year 1904, a period when plague was especially severe in the adjacent district of Bellary and Anantapur.

The districts which have been more severely affected by plague and which still remain to be considered, number eight. They were all infected for the first time between the years 1898 and 1903 and in them nearly 97 % of the plague deaths have occurred. From Table I it will be seen that the Bellary district has been the most severely infected in the Presidency.

TABLE I. *Average annual death rate from plague per mille of the affected districts for thirteen years from 1898 to 1910.*

District	Total plague deaths	Population	Average annual death rate per mille from plague
Bellary	31,510	947,214	2·56
Nilgiris	1,403	111,437	·97
Salem	13,312	2,204,974	·46
Coimbatore	12,006	2,201,752	·42
Anantapur	3,507	788,254	·34
South Canara	3,010	1,130,105	·20
North Arcot	3,550	2,207,712	·12
Kurnool	1,042	872,055	·09

Here nearly half of the total deaths from plague in the Presidency have been notified. Next to Bellary in the severity of its epidemics although with a comparatively small death-roll owing to its small population, comes the Nilgiri district. In another group may be

classed the Salem and Coimbatore districts each with from 12,000 to 13,000 deaths from plague. In a fourth group may be placed Anantapur, South Canara and North Arcot with between 3000 and 4000 deaths each, and lastly Kurnool with little over 1000 deaths.

Tables No. II and III give respectively the total number of deaths and death rate per mille per annum in each of the districts mentioned above from the year 1898 to the end of the year 1910. These tables show that in the year 1904 plague caused the greatest havoc, especially in the Bellary district where more than 10,000 persons died of the disease.

They also show that, in the eight districts, plague has been more or less continuously present since 1898 but that during recent years from 1905 onwards the epidemics have become less severe. It has been pointed out that there has been little tendency for the outward spread of the epidemics from the eight severely infected districts to the surrounding parts of the Presidency; such extensions as have occurred beyond the districts infected early in the year have been few, of short duration and irregular in distribution both as regards time and space. The total plague mortality other than in the eight districts mentioned above has been so small as to be almost negligible.

V. *The influence of climate on the distribution of plague in the Madras Presidency.*

We can now proceed to show, as we have incidently demonstrated above in discussing the distribution of plague in the less severely infected districts, that in the severely infected districts plague has tended to be more severe and more widely spread in those areas in each district where the temperature is lower and the humidity higher than the average. It must be clearly understood, however, that we do not for a moment suggest that the climate in any part of the district is the sole factor which influences the spread of plague. We are fully alive to the importance, for example, of facilities for communication between infected and uninfected areas which in turn depends on such factors as the density of the population in a given area, the proximity of the places to railways and roads as well as the nature and the extent of the occupations and trades of the people inhabiting the area.

We will not overlook these factors where we think they are of importance, but desire here to study, especially, the distribution of plague so far as it is correlated with variations in the climate of each

TABLE II. *Annual number of deaths in certain districts in Madras Presidency from 1898 to 1910.*

District	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	Totals	Total for epidemic years
Bellary	125	173	64	1,009	7,354	5,896	10,751	3,490	325	1,583	587	18	135	31,510	31,510
Salem	109	569	573	1,553	1,668	3,132	1,313	481	99	923	412	302	2,178	13,312	13,312
Coimbatore	1	37	—	44	496	1,184	4,309	333	30	10	1,183	2,973	1,407	12,007	12,006
North Arcot	54	562	15	399	114	887	1,116	255	24	47	38	20	34	3,565	3,560
Anantapur	205	362	2	9	89	677	1,585	381	3	34	174	—	1	3,522	3,507
South Canara	1	—	1	1	993	365	382	525	153	126	216	98	152	3,013	3,010
Nilgiris	—	1	—	—	1	601	81	200	45	49	143	210	31	1,405	1,403
Kurnool	8	140	—	3	55	491	270	78	3	.1	—	—	—	1,049	1,042

TABLE III. *Annual death rate from plague per 10,000 in affected districts from 1898 to 1910.*

	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910
Anantapur	2.6	4.5	—	—	1.1	8.5	20.1	4.8	—	.4	2.2	—	—
Bellary	1.3	1.8	.6	10.5	77.5	62.2	113.5	36.8	3.4	16.7	6.1	.1	1.4
Coimbatore	—	.1	—	.2	2.2	5.3	19.5	1.5	.1	—	5.3	13.5	6.3
Cuddapah	—	—	—	—	—	—	2.1	.1	—	*	—	—	—
Kurnool	*	1.6	—	—	.6	5.6	3.0	.9	—	—	—	—	—
Madras City	—	—	—	—	—	—	—	.4	1.1	—	—	—	—
Madura	—	—	—	—	—	—	*	—	—	—	—	—	2.9
Malabar	—	—	—	—	—	—	—	—	.3	.2	2.1	.7	.2
Nilgiris	—	—	—	—	—	—	—	—	4.0	4.4	12.8	18.8	2.7
North Arcot	.2	2.5	—	1.8	.5	4.0	5.0	1.1	.1	.2	.1	*	.1
Salem	.4	2.5	2.6	7.0	7.5	14.1	5.9	2.1	.4	4.1	1.8	1.3	9.8
South Canara	—	—	—	—	8.7	3.2	3.3	4.6	1.3	1.1	1.9	.8	1.2

\* Plague affected, but less than one death per 100,000.



district. In this connection we again desire to draw attention to the papers published by the Commission on the bionomics of the rat flea (*X. cheopis*) (see *Journal of Hygiene*, Plague Supplement II, 1912, p. 317, also Plague Supplement III, p. 613), which show that comparatively small variations in the temperature and humidity of the atmosphere in which these insects are kept has a marked effect in increasing or shortening the period they are able to remain alive away from their hosts.

The districts in which we have now to consider the distribution of plague are enumerated in Tables I, II and III and from Map 2. It will be observed that they form an almost complete circle round the Mysore State.

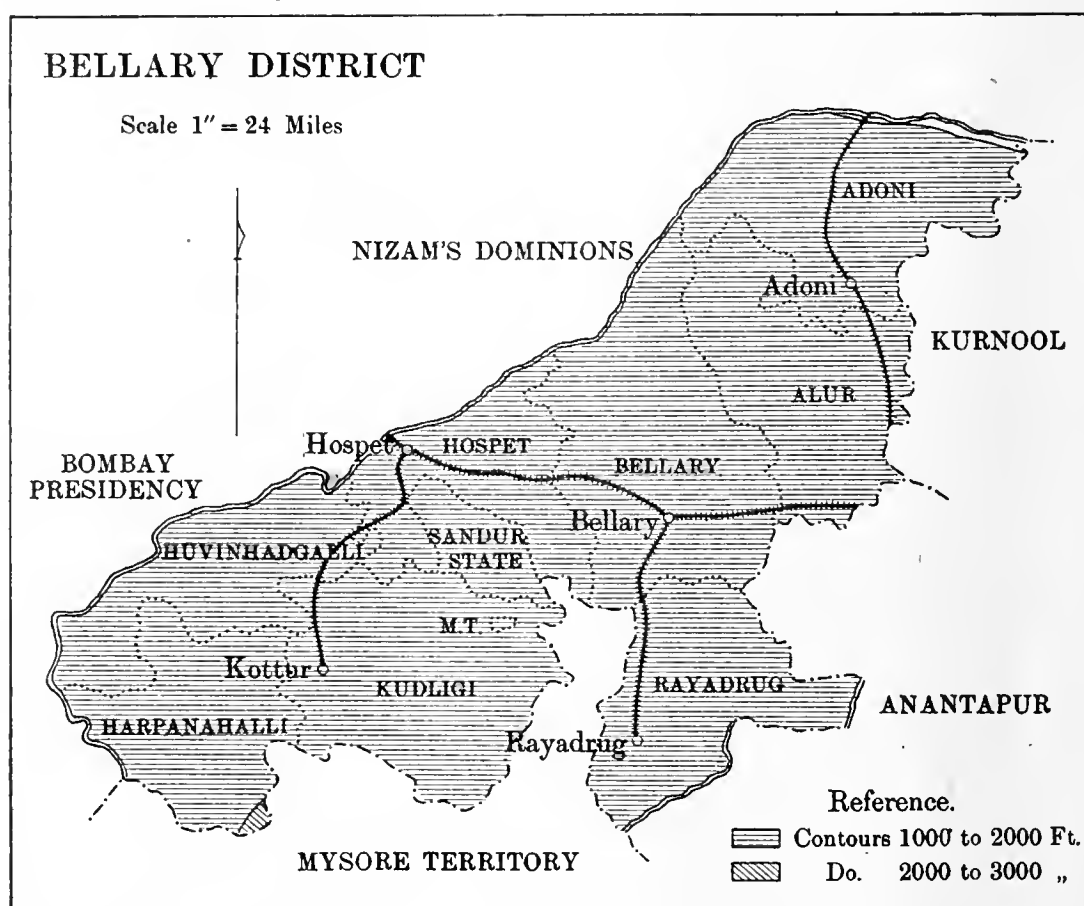
*The Bellary, Kurnool and Anantapur districts.*

The Bellary district is a flat fairly densely populated plain which lies to the north of the Mysore State sloping gradually upwards from an elevation of about 1000 feet above the sea in the east to 1500 feet in the west. This district is so situated in respect to the monsoon current that it is subject to periodic years of drought and famine, the average rainfall being only 19 inches. The advancing South-West monsoon current barely reaches the eastern portion of the district while the retreating current which recurves and is determined on the east coast, in some years hardly extends so far north and west as the Bellary district. The climate of these districts therefore varies considerably from year to year; it depends on the strength of the monsoon currents which may or may not extend into it, so that the Chart (No. 18) illustrating the average climatic conditions does not indicate satisfactorily the climate in any single year.

When considering Tables II and III we pointed out that the year 1904 was the most severe year of plague in the Bellary district, in that year more than 10,000 persons died of the disease; it is, therefore, significant to note that the winter of the years 1903—04 was an abnormal year in respect to the climate of this district. The average mean daily temperature for the months December and January was as low as 73° F. and the average daily humidity from November to January was unusually high, the average of the daily readings for the months of November and January being 80%. These are conditions suitable for the diffusion of plague.

The accompanying Map, No. 3, shows the general contour of the district, its divisions into taluqs, and its railway lines. On the western border the district adjoins the Dharwar district of the Bombay

Presidency and the Nizam's Dominions. From the Dharwar district, one of the most severely plague-infected parts of the Bombay Presidency, a railway leads through Hospet to Bellary, while from Raichur, in the Nizam's Dominions, a railway passes through Adoni to Madras. The facilities for communication with infected areas therefore are great and the following Table IV shows that the Hospet and Bellary taluqs have been most severely affected.



Map 3

TABLE IV. *Plague deaths in each taluq of the Bellary district, 1902—1910.*

Taluq	Plague deaths, 1902—1910	Population, 1901 census	Average annual deaths per mille
Bellary	10,542	193,401	6·057
Alur	1,706	98,568	1·924
Adoni	4,901	178,784	3·047
Rayadrug	2,615	82,789	3·513
Hospet	5,774	101,947	6·295
Haddagalli	2,741	92,094	3·310
Harpanahalli	996	95,646	1·157
Kudligi	1,052	103,985	1·125
Bellary taluq minus Bellary town	9,333	135,154	6·908
Adoni taluq minus Adoni town	2,542	148,308	1·714

These taluqs are the nearest to and are in direct communication with the badly infected Dharwar district, a fact which suggests that the severity of plague in this district to some extent depends upon its proximity to and facilities for communication with this infected area. Additional support is lent to this view when it is recognised that the climate of the Kurnool district differs but little from that of Bellary (compare Charts 17 and 18). Up to the year 1910, however, 31,500 deaths from plague have been recorded in Bellary while only 1049 have been notified in Kurnool and nearly 800 of these have occurred in the Patticonda taluq, the most westerly taluq of that district and adjoining Bellary. We must note however that free communication with the eastern part of the Kurnool district is to some extent interrupted by the Eramalai and Nalamalai hills which cross the district from north to south and again that the western part of the Bellary district is considerably more elevated than the eastern part of the district, the climate gradually tending to approximate to that of the Dharwar district as one passes from the east to the west of the Bellary district.

The Anantapur district lies to the south of the Bellary district, the least elevated portion of this district (see Map No. 4) is at Tadpatri in the north-east of the district. From this point the land slopes upwards to Gooty in the north-west and in the south rises gradually to the Mysore plateau.

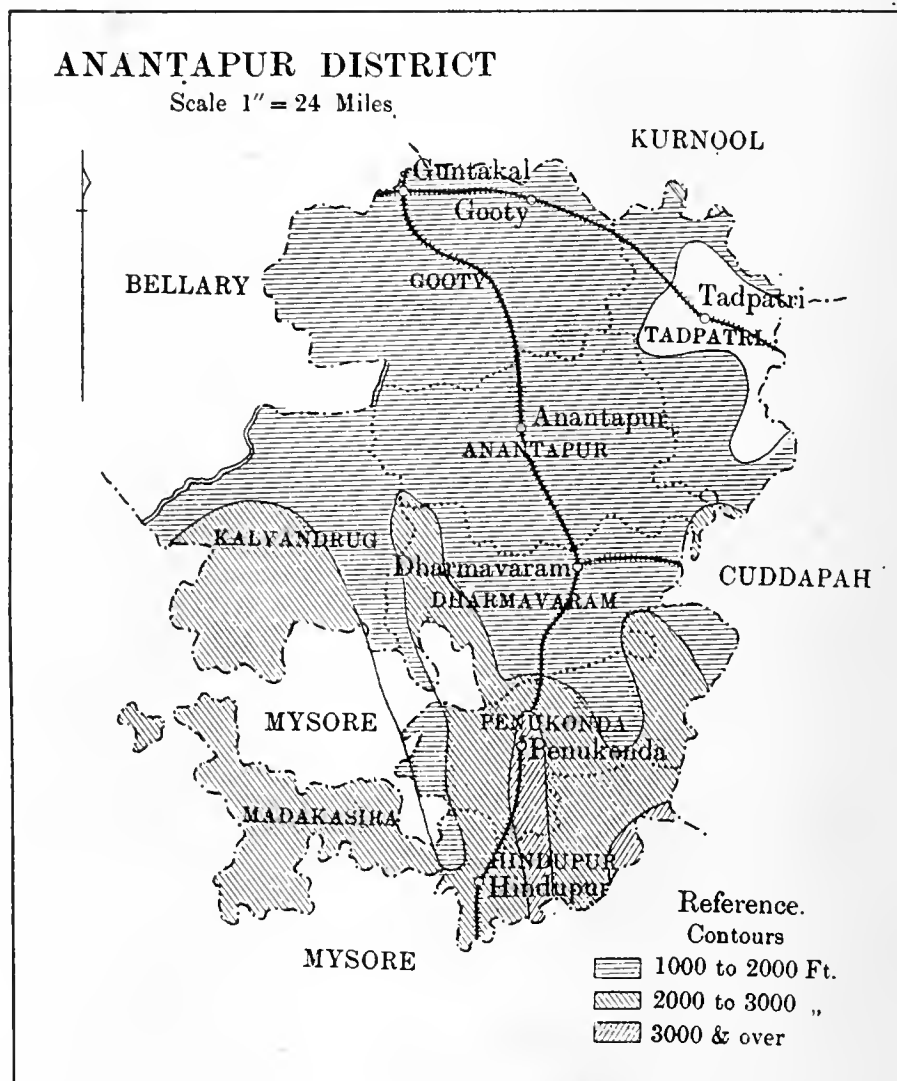
Table V, giving the distribution of plague deaths to the taluq divisions, shows that plague has been most severe in the Gooty taluq.

TABLE V. *Plague deaths in each taluq of the Anantapur district, 1902—1911.*

Taluq	Population, 1901 census	Plague deaths, 1902—1911	Average annual plague deaths per mille
Anantapur	108,731	148	·136
Gooty	156,155	1,511	·967
Tadpatri	109,421	6	·005
Penukonda	92,482	167	·180
Dharmavaram	70,943	66	·093
Hindupur	92,088	734	·797
Madakasira	81,457	201	·246
Kalyandrug	76,977	173	·224

Gooty adjoins the Bellary district and is connected with it directly by two lines of railway, one passing through the Adoni taluq of the Bellary district to the Nizam's Dominions, the other through Bellary and Hospet taluqs to the Dharwar district of the Bombay Presidency. A number

of deaths from plague have also occurred in the Hindupur district in the extreme south of the district which lies at an elevation and enjoys a climate approximating to that of the Mysore plateau. The Tadpatri taluq which is the lowest-lying and the hottest part of the district, although only one and a half hours distant by rail from Gooty, has practically escaped the disease, while the more elevated and cooler taluqs show a proportionately greater number of deaths from plague.

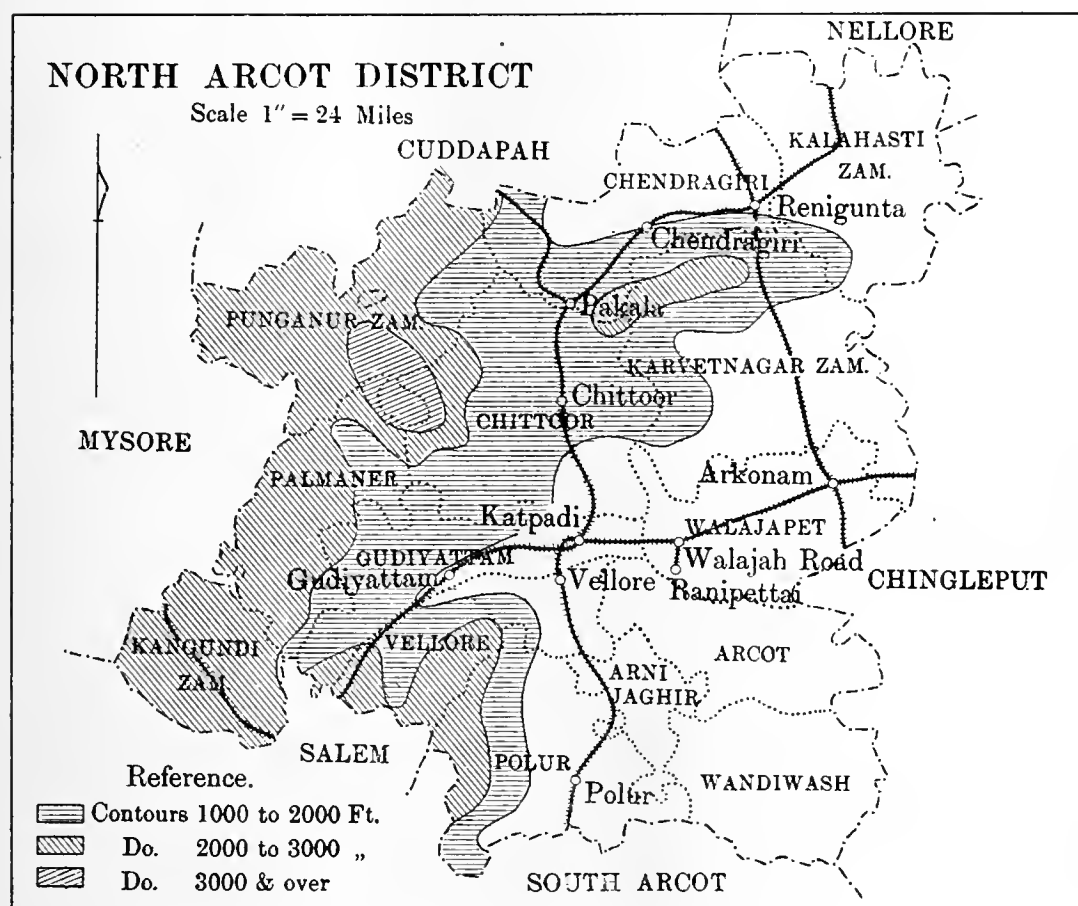


Map 4

*The North Arcot, Salem and Coimbatore districts.*

These districts adjoin the south-eastern boundary of the Mysore State, small portions of each district lie on the Mysore plateau and the land tends to fall more or less gradually from these elevated and cooler portions of the district to the low-lying hot plains on the east and south.

A characteristic feature of the distribution of plague in these districts is the intensity and persistence of plague in the villages in the higher lying areas. Only occasional severe epidemics occur in the municipal towns of the less elevated areas where plague in the villages hardly exists. In the North Arcot district for example (see Map No. 5), besides the elevated somewhat isolated and sparsely populated Punganur and Kangundi zemindaries and the Palmaner taluq, small portions of the



Map 5

Vellore and the Gudiyattam taluqs lie at the elevation of about 1000 feet above the level of the sea. A railway line passes through this high land and it connects the severely plague smitten city of Bangalore in Mysore with Madras. Plague has been more severe in these parts of the North Arcot district, where climate and facilities for communication with infected areas are most favourable (see Table VI). This table also shows that Vellore town has suffered from one or two epidemics of plague.

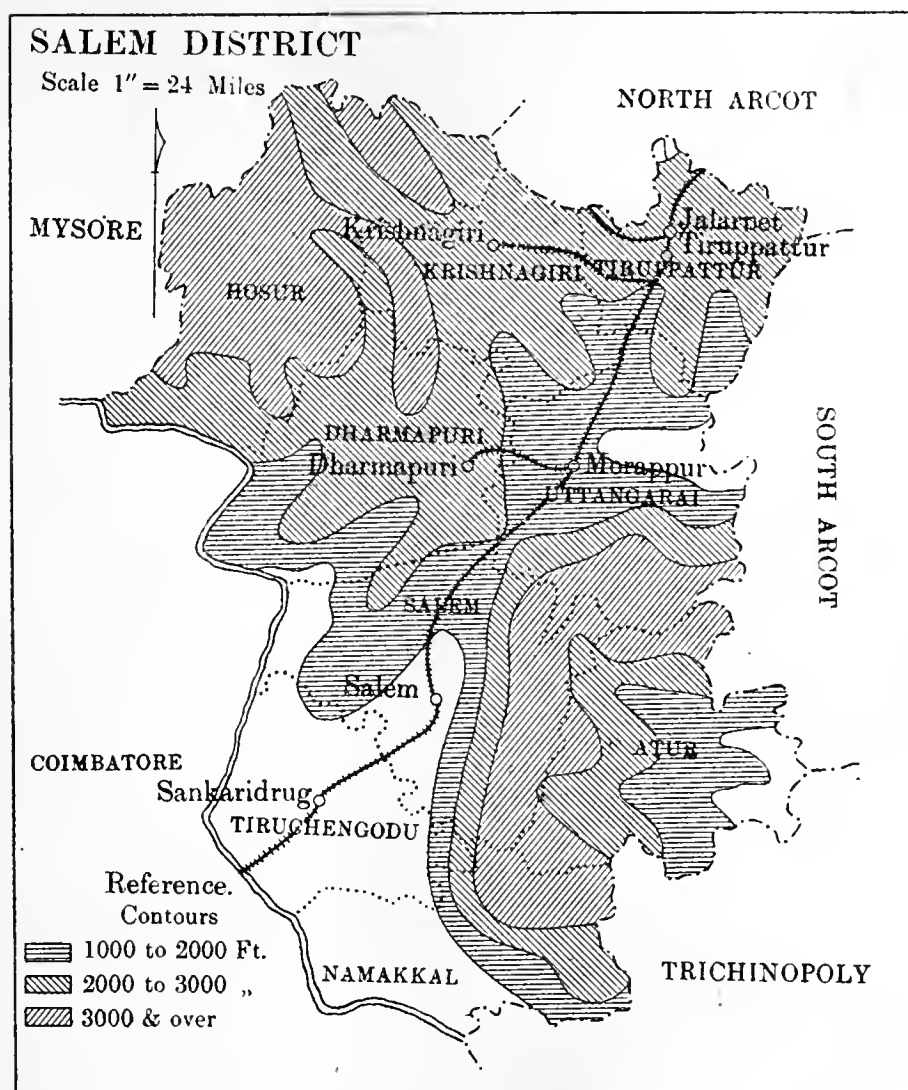
TABLE VI. *Plague deaths in each taluq of the North Arcot district, 1900—1910.*

Taluq *					Population, 1901	Plague deaths, 1900—1910	Average annual plague death-rate per mille
Vellore	...	...	...	...	200,541	1,557	·706
Gudiyattam	...	...	...	...	195,665	439	·204
Chittoor	...	...	...	...	209,868	144	·062
Polur	...	...	...	...	155,673	129	·075
Palmaner	...	...	...	...	51,575	40	·70
Arcot	...	...	...	...	180,564	96	·048
Wandiwash	...	...	...	...	185,252	18	·008
Chandragiri	...	...	...	...	113,550	5 (not indigenous)	—
Walajapet	...	...	...	...	221,812	40 (25 indigenous)	·010
Zemindaries							
Punganur	...	...	...	...	96,852	10 (not indigenous)	—
Kangundi	...	...	...	...	64,542	96	·092
Karvetnagar	...	...	...	...	341,240	17 (not indigenous)	—
Kalahasti	...	...	...	...	94,132	11 (not indigenous)	—
Arni	...	...	...	...	96,542	307	·195
Vellore taluq minus Vellore town					157,004	759	·438
Vellore town	...	...	...	...	43,537	798	1·667

Again, in the Salem district, the Hosur taluq, which from Map 16 will be seen to be situated at an elevation of more than 3000 feet on the Mysore plateau, has suffered severely and almost continuously from plague, see Tables VII and VIII.

TABLE VII. *Table showing the number of deaths from plague from 1902—1911 in the taluqs of the Salem district.*

Taluq	Plague deaths, 1902—1911	Population, 1901 census	Average annual plague deaths per mille
Atur	549	199,475	·275
Dharmapuri	297	206,030	·144
Hosur	4,003	184,971	2·164
Krishnagiri	444	175,300	·253
Salem	2,920	470,181	·621
Tiruchengodu	115	289,717	·039
Uttangarai	10	159,419	·006
Namakkal	14	313,895	·004
Tiruppattur	4,508	205,986	2·140



Map 6

TABLE VII (a). *Deaths from plague in Hosur taluq.*

	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	Totals
January	12	18	135	141	23	60	1	26	84	50	6	13	569
February	—	20	59	139	14	57	—	26	69	32	4	16	436
March	3	23	36	36	5	9	—	18	53	12	—	39	234
April	1	11	14	6	6	10	4	35	5	3	3	24	122
May	—	10	—	—	17	—	5	12	10	1	—	24	79
June	—	17	6	—	27	1	—	32	5	—	—	44	132
July	—	14	25	6	48	1	—	64	21	10	1	75	265
August	23	33	19	16	95	1	1	124	16	21	5	109	463
September	9	24	39	24	127	1	7	200	15	28	1	116	591
October	2	33	27	18	144	—	51	127	15	20	10	100	547
November	12	59	42	18	92	—	8	131	42	7	3	75	489
December	13	174	176	15	56	1	35	111	37	1	7	63	689
Totals	75	436	578	419	654	141	112	906	372	185	40	698	—

## 722 *Epidemiological Observations in Madras Presidency*

Table VIII shows that some severe epidemics of plague have occurred in the municipalities of this district, but the disease has caused few deaths in the villages except in Hosur taluq. Atur, Krishnagiri and Dharmapuri taluqs at moderate elevations had suffered to a greater extent than the more low-lying and hotter Uttangarai, Namakkal and Tiruchengodu taluqs.

TABLE VIII. *Annual plague deaths in the taluqs and municipalities of the Salem district each year from 1902—1911.*

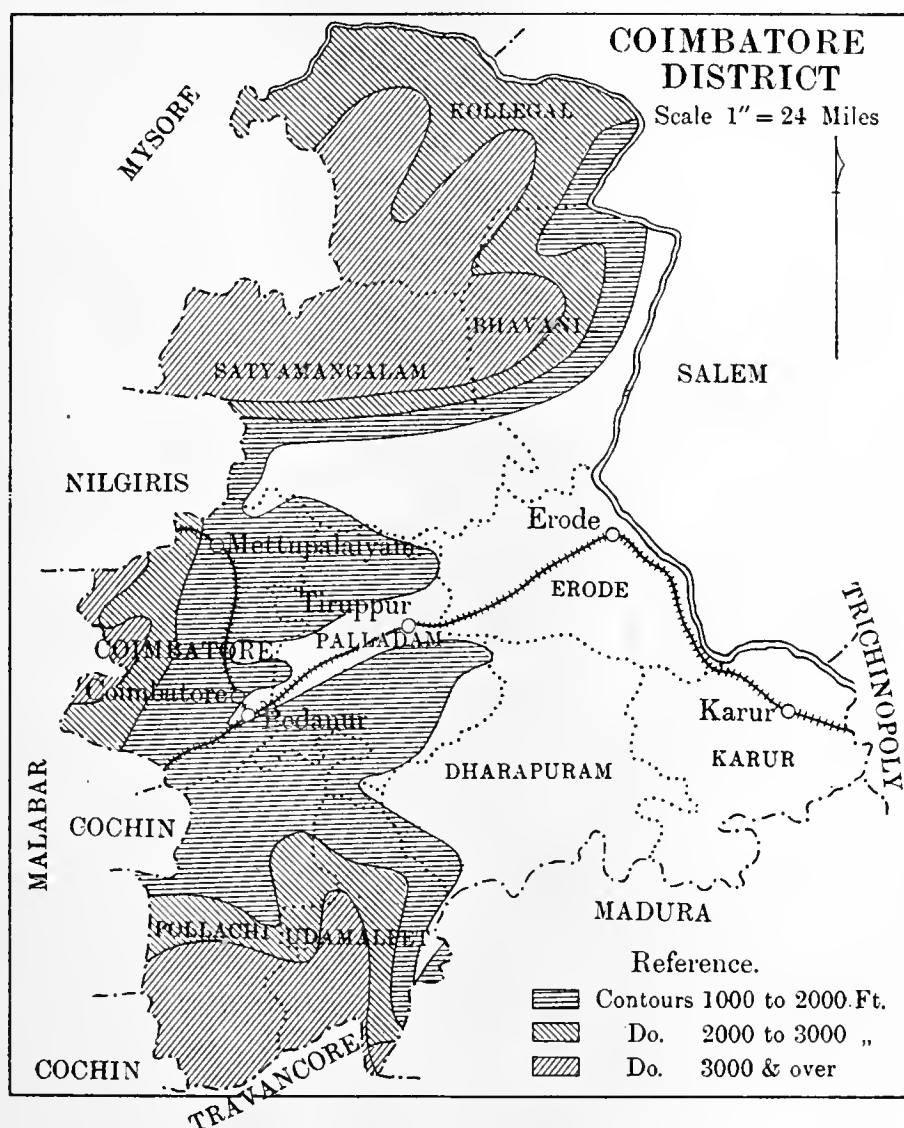
Taluq	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911
Atur	—	—	—	—	—	—	—	—	151	385
Dharmapuri	8	5	—	—	—	—	—	2	2	280
Hosur	578	378	660	140	92	906	313	185	40	711
Krishnagiri	18	199	60	3	1	—	25	105	2	31
Omalur	—	—	—	—	—	—	—	—	162	181
Salem	1	2	2	1	—	1	64	—	353	568
Tiruchengodu	—	—	30	—	—	—	—	2	31	52
Uttangarai	—	4	—	1	1	—	—	1	2	1
Tiruppattur	90	514	94	16	—	6	9	—	21	—
Namakkal	—	—	3	—	—	—	—	—	11	—
Municipalities										
Salem	—	2	2	—	—	—	—	1	1,397	183
Vaniambadi	961	1,365	10	2	—	—	1	1	1	—
Tiruppattur	12	617	443	326	5	3	—	1	10	—

From Map 7 it will be seen that the Kollegal taluq and part of the Satyamangalam and Bhavani taluqs of the Coimbatore district are situated at an elevation of more than 2000 feet. They therefore possess a climate approximating to that of Mysore. The Beligiri-rangau and the Bargur hills run across the higher portions of the Satyamangalam and Bhavani taluqs and extend into the Kollegal taluq. The hill portions of Satyamangalam and Bhavani and the greater part of the Kollegal taluq are covered with forests and are sparsely inhabited. The portion of the Kollegal taluq, however, adjoining the Mysore State forms a well-cultivated and populated plain.

In the west of the district there is an area at an elevation of about 1000 feet which is situated opposite the Palghat Gap. This area includes the taluqs of Coimbatore and Pollachi which receive the cool moist westerly breezes which blow from the sea through the gap. The taluq of Dharapuram also receives some benefit from these breezes but it is less marked here as the gap widens out. In the southern portion the high contour shown on the map represents the forest-covered Anamalai hills.



From Tables IX and X it will be seen that the Kollegal taluq, relatively to its population, has suffered severely from plague and that epidemics have occurred in the municipal town of Coimbatore and in some of the surrounding villages of this taluq. The low-lying taluq of Dharapuram has had some plague in its villages but, as has been



Map 7

pointed out, the climate of this taluq is considerably modified and cooled by the breezes which reach it through the Palghat Gap. The low-lying taluqs of Erode and Karur and the low-lying and populated parts of the Satyamangalam and Bhavani taluqs have suffered very little from plague.

Only a word or two remains to be said of the plague in the Nilgiri and South Canara districts. The former district situated in the midst

## 724 *Epidemiological Observations in Madras Presidency*

TABLE IX. *Annual deaths from plague in the taluqs and municipal towns of the Coimbatore district, 1902—1911.*

Taluq	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911
Coimbatore	—	—	1,122	156	13	—	6	1,071	310	577
Bhavani	—	—	14	8	—	—	3	1	1	13
Dharapuram	—	—	71	—	—	—	16	1	34	1
Kollegal	—	—	2,060	97	16	1	1,151	657	234	159
Palladam	—	—	841	20	—	—	1	84	56	95
Satyamangalam	—	—	24	3	—	6	10	21	16	1
Karur	—	—	1	—	—	—	—	8	1	—
Avanashi	—	—	—	—	—	—	—	—	—	25
Pollachi	—	—	62	25	—	—	—	5	305	14
Udamalpet	—	—	148	9	—	2	9	—	304	163
Erode	—	—	27	2	—	1	1	8	1	4
Municipal towns										
Coimbatore	—	—	203	5	1	—	—	1,051	55	767
Erode	—	—	76	8	8	—	—	85	11	6
Karur	—	—	—	—	—	—	—	3	—	—
Zemindaries										
Pollachi	—	—	6	—	—	—	—	—	57	9
Udamalpet	—	—	—	—	—	—	—	—	1	1

TABLE X. *Total plague deaths in each taluq of the Coimbatore district, 1904—1911.*

Taluq	Plague deaths, 1904—11	Population, 1901 census	Average annual plague deaths per mille
Coimbatore	5,377	330,684	2·017
Kollegal	4,375	96,563	5·667
Erode	238	275,460	·108
Bhavani	40	145,982	·034
Palladam	1,097	300,904	·455
Dharapuram	1,231	271,127	·566
Karur	13	220,843	·007
Satyamangalam	91	214,101	·053
Pollachi	483	195,608	·308
Udamalpet	635	150,480	·527

of lofty mountains is of small area and has a comparatively small and scattered population. Owing to the great variation in elevation in the different parts of the district, the climatic features are very varied. At the higher elevations (8000 feet) the climate resembles that of the temperate zone rather than the tropics, while in the valleys at the foot of the mountains essentially tropical conditions exist. Plague has been persistently present in the district since it was first introduced in 1903; the absence of well-marked hot weather no doubt conduces to this end. Relatively to the population the epidemics have been severe and the disease has appeared in the towns, villages and hamlets alike despite

the very indifferent means of communication which exist between one place and another in such a mountainous country. The cool climate favours the prolonged existence of the rat flea and would thus assist in the transfer of infection from place to place.

Plague in South Canara has been almost altogether confined to Mangalore City where the disease has appeared chiefly in the overcrowded and dirty portions inhabited by Moplas. Plague practically never occurs in the out-lying portions of the town where the houses are more scattered and isolated nor in the houses which are found distributed at intervals in the midst of coconut groves in the surrounding country. The other features of plague in this town are that the epidemics have, individually, never been as severe as in most other towns in India nor yet have they died out completely during the hot weather which is so marked a feature of plague epidemics in the more northern parts of India. These peculiarities, we think, can be accounted for by the fact that the climate of the district is never very favourable for nor yet quite unsuited to the development of plague epidemics. An examination of Chart 11 will show that there is very little variation in temperature throughout the year. The mean temperature remains about 80° F. both in summer and winter, indeed, the most curious feature of the climate is that the summer and autumn are cooler than the winter and spring. The largest number of cases of plague have occurred in August and September, at a time when the humidity is high.

VI. *Rat and flea prevalence in different parts of  
Madras Presidency.*

For the purpose of observing the rat and flea prevalence under the varying climatic conditions in different parts of the Presidency, four laboratories were established in the middle of the year 1911 in places showing these varying conditions and also a varying incidence of plague.

These laboratories were stationed at :

(1) Denkanikota representing the Mysore Plateau and conditions favourable to endemic plague.

(2) Coimbatore representing the peculiar conditions in the area opposite the Palghat Gap.

(3) Vaniambadi representing the central districts at an elevation of 1000 feet where occasionally large outbreaks have occurred in the municipalities.

(4) Madura representing the hot southern districts which have escaped plague.

Observations were carried out in each of the four places for a complete year, rats being trapped systematically and flea counts made on them daily. The methods used were exactly similar to these which the Commission had previously employed in Poona, Belgaum and other parts of India.

The following is an account of the observations in these stations :

*Observations in Denkanikota.*

When we decided to make observations in a place situated on the Mysore Plateau our choice first fell upon Hosur town in the Hosur taluq of the Salem district. But when we reached that place we found that an epidemic of plague had broken out and many of the inhabitants had left their homes and closed their houses so that trapping rats in the houses of the town was not practicable. Denkanikota was therefore chosen as the nearest village of suitable size representing much the same conditions.

Denkanikota is a village of 5732 inhabitants, situated in the Hosur taluq of the Salem district at an elevation of 3020 feet above sea-level. It is 16 miles from Hosur town and 41 miles from Bangalore by road. The nearest railway station is Malur 38 miles distant. Communications with the rest of the Salem district are poor. The village has little trade and no manufacture, most of the people being engaged in agriculture.

The houses are of the poorest description, most of them being built of mud walls, with bamboo rafters, covered with a roof of several layers of country tiles. There are a few houses of better description.

The village first suffered from plague in 1901, and was again infected in 1902, 1906, 1907, 1908 and 1909.

Operations were commenced here on 11th August 1911 and were carried on till 15th August 1912. A hundred traps were set daily for the greater part of that period. Readings of temperature and humidity were taken from 16th September onwards and the results of this work are shown on the accompanying tables and charts (Tables XI and XII and in Chart 27).

Table XI shows that owing to the small size of the village and relatively large number of traps set, the number of rats caught per 100 traps set fell rapidly as the period extended during which trapping was practised, so that from the month of March onwards the number of rats

caught was small. Flea counts made on few rats are not always reliable, but the regularity of the curve of flea prevalence shown on Chart 27 demonstrates that in the present instance this possible source of error was of little moment. The greatest fortnightly average number of fleas per rat was 14·9 in August. This period is associated with a marked rise in the number of deaths from plague in the Hosur taluq as may be noted by a reference to Table VII (a). The smallest average number of fleas per rat was observed in May, when the number of plague deaths in the taluq is at its lowest. An examination of Chart 27 and Tables XI and XII will show that the largest number of fleas are found when the temperature is about 75° F. and the humidity over 80 %.

TABLE XI. *Rat and flea prevalence. Fortnightly average.*

1911 Fortnight ending	Rats per 100 traps	Fleas per rat	1912 Fortnight ending	Rats per 100 traps	Fleas per rat
19 August	27·5	14·9	3 February	5·4	8·4
2 September	18·2	12·2	17 „	5·3	6·2
16 „	13·3	12·5	2 March	3·9	5·9
30 „	9·9	10·4	16 „	3·0	7·9
14 October	8·6	10·6	30 „	3·0	6·7
28 „	4·3	11·8	13 April	2·1	5·3
11 November	13·4	9·9	27 „	3·5	6·4
25 „	5·3	9·6	11 May	2·4	3·7
9 December	5·5	8·1	25 „	12·4	3·1
23 „	7·3	8·8	8 June	4·5	4·5
			22 „	5·3	6·1
1912			6 July	6·2	5·5
6 January	3·8	9·7	20 „	6·8	7·5
20 „	6·0	9·0	3 August	2·6	10·0

TABLE XII. *Fortnightly mean temperature and humidity.*

1911 Fortnight ending	Tempera- ture	Humidity	1912 Fortnight ending	Tempera- ture	Humidity
30 September	75·5	82·0	2 March	78·2	61·2
14 October	76·5	82·5	16 „	79·9	55·5
28 „	74·0	81·5	30 „	82·6	60·7
11 November	74·5	78·0	13 April	85·4	82·7
25 „	72·5	84·5	27 „	85·2	67·4
9 December	73·0	*	11 May	84·7	68·0
23 „	70·0	*	25 „	85·1	73·0
1912			8 June	82·5	74·7
6 January	69·8	*	22 „	79·2	79·7
20 „	70·5	70·5	6 July	79·1	75·9
3 February	72·4	62·9	20 „	78·5	80·6
17 „	75·6	76·5	3 August	76·8	82·9

\* Humidity figures not reliable; apparatus out of order.

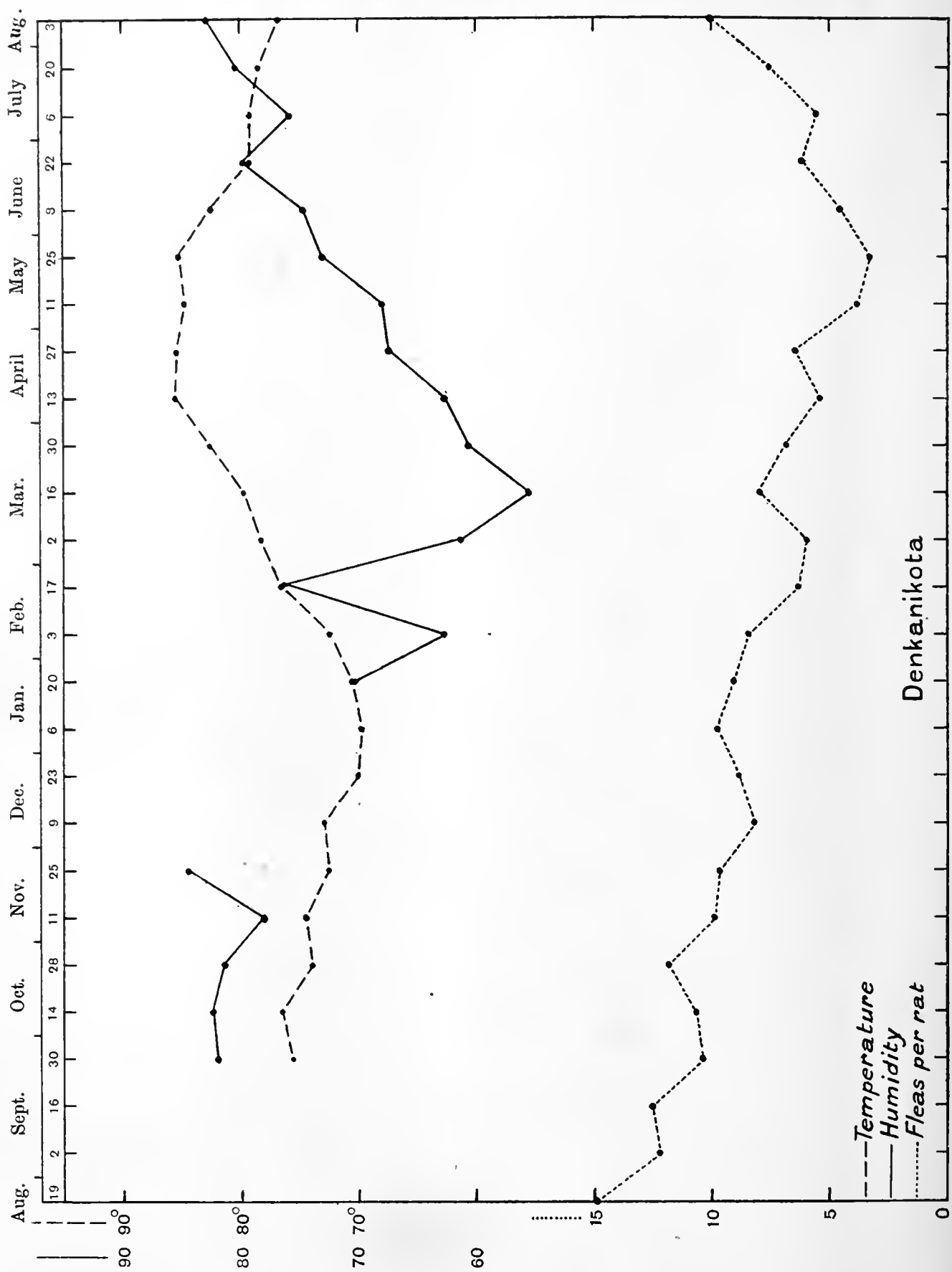


Chart 27

*Observations in Coimbatore.*

Coimbatore is the headquarter town of the district of the same name. It is situated at the mouth of the Palghat Gap on its eastern side and lies at an elevation of 1340 feet above sea-level. The railway line from Podanur to Mettapulliam and the Nilgiri Hills passes through it. Through Podanur, which is four miles off, Coimbatore communicates with the west coast towns on the one side and with the Central districts and Madras City on the other. Mysore State can be reached by a circuitous journey through Erode and Jollarpet junctions to Bangalore. There is road communication with Pollachi, Mettapulliam and Satyamangalam.

The population of Coimbatore in 1901 was 53,080 and according to the census of 1911 was 40,007. Plague was however present in the town when this second census was taken.

It is an important industrial and trading centre. The industries include cotton spinning (both hand and mechanical loom weaving), coffee-curing, sugar-refining, etc. The town is for the most part badly built and there are several congested areas in it. The houses are of the type common in the Central districts—mud or brick walls with roofs of country tiles in several layers. The proportion of houses of a better type is small.

Coimbatore has a mild and pleasant climate which has already been commented upon (page 701 and Chart 19). The normal mean temperature falls below 80° F. at an earlier period than in most other parts of the Presidency. The hot weather is very short and the temperature is never very high (see Chart 19).

The town has suffered from epidemics of plague in the years 1903, 1904, 1909, 1910, 1911 and 1912. The deaths in these years being 571, 761, 1, 101, 55 and 764 respectively.

The seasonal prevalence of plague is earlier than in other parts of the Presidency, the plague death rate reaching a considerable height in August and September. Infection is usually supposed to be derived from the Nilgiris. Observations were commenced in Coimbatore on 1st May 1911, 150 to 300 traps being set daily and flea counts made on the rats collected. The results are shown in the accompanying tables and in Chart 28.

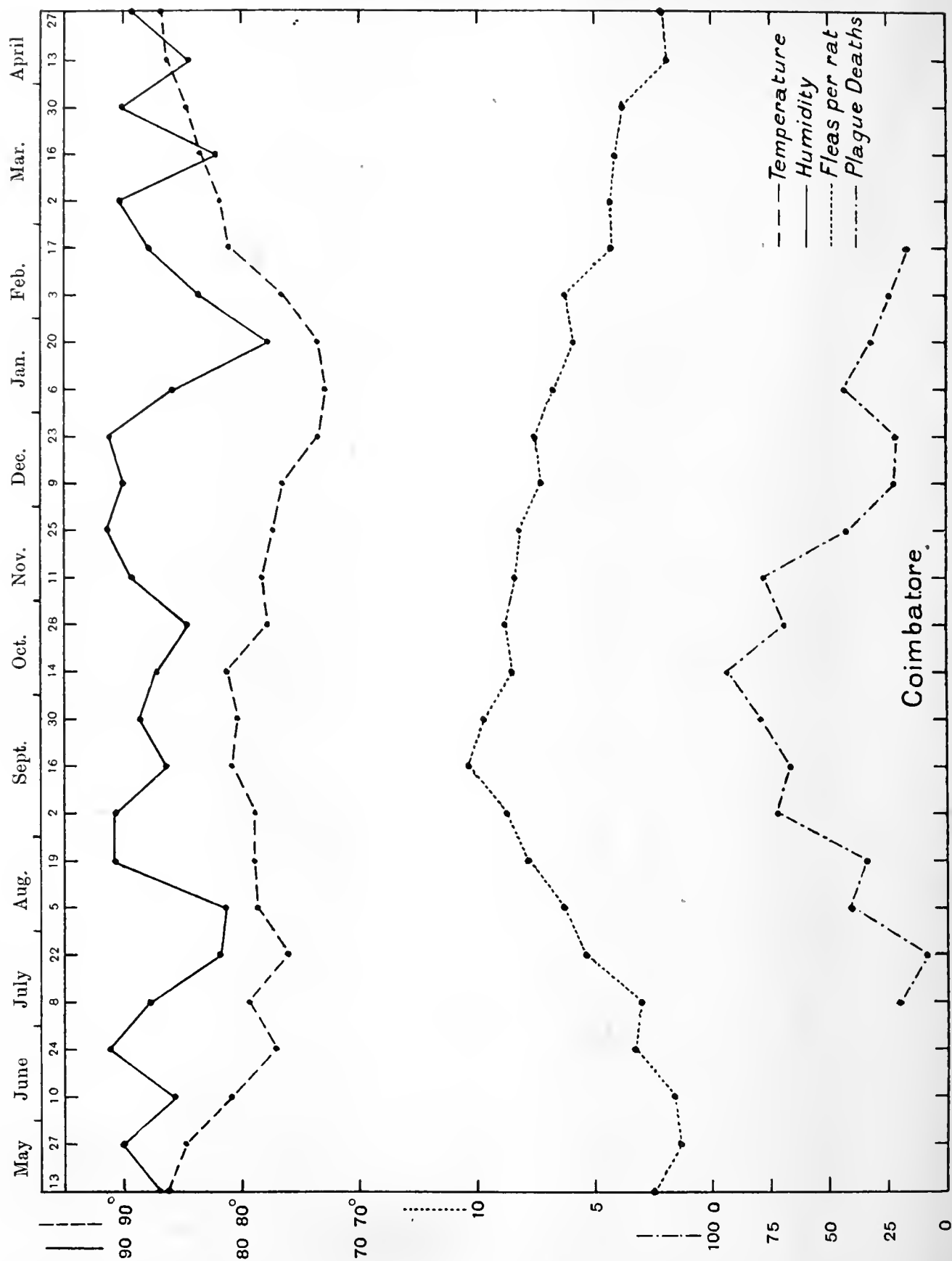


Chart 28



TABLE XIII. *Rat and flea prevalence in Coimbatore.  
In fortnightly periods.*

1911 Fortnight ending	Rats per 100 traps	Average fleas per rat	1911 Fortnight ending	Rats per 100 traps	Average fleas per rat
13 May	7.7	2.5	9 December	3.3	7.2
27 „	9.7	1.4	23 „	2.6	7.5
10 June	6.0	1.6			
24 „	6.7	3.3	1912		
8 July	5.9	3.0	6 January	2.1	6.7
22 „	5.5	5.4	20 „	1.6	5.9
5 August	5.2	6.3	3 February	2.1	6.2
19 „	6.6	7.8	17 „	1.2	4.2
2 September	11.6	8.7	2 March	2.2	4.3
16 „	5.9	10.4	16 „	1.8	4.1
30 „	4.6	9.7	30 „	2.6	3.8
14 October	5.1	8.5	13 April	2.6	1.9
28 „	4.1	8.8	27 „	4.0	2.1
11 November	4.8	8.4	11 May	2.9	2.1
25 „	2.9	8.1			

TABLE XIV. *Statement showing average temperature and humidity  
for fortnightly periods at Coimbatore.*

(Figures taken from Government Meteorological Reports.)

1911 Fortnight ending	Average temp.	Average humidity	1911 Fortnight ending	Average temp.	Average humidity
13 May	86.4	86.9	9 December	76.4	90.0
27 „	84.8	90.0	23 „	73.4	91.1
10 June	80.9	85.6			
24 „	27.0	91.1	1912		
8 July	79.3	87.8	6 January	72.8	85.9
22 „	76.0	81.8	20 „	73.5	77.8
5 August	78.6	81.2	3 February	76.5	83.7
19 „	78.9	90.6	17 „	81.0	87.9
2 September	78.9	90.6	2 March	81.9	90.2
16 „	80.9	86.3	16 „	83.4	82.1
30 „	80.3	88.6	30 „	84.7	90.0
14 October	81.2	87.1	13 April	86.2	84.4
28 „	77.9	84.6	27 „	86.8	89.1
11 November	78.1	89.2	11 May	—	—
25 „	77.3	91.3			

The effect of the epizootic is shown by a fall in the number of rats caught per 100 traps set (see Table XIII), for very few rats were captured in proportion to the number of traps laid after the epizootic had continued for some time, *i.e.* during the whole period of our observations in the year 1912. It is interesting also to observe that the height of the epidemic was attained shortly after the fleas became most

## 732 *Epidemiological Observations in Madras Presidency*

numerous on the rats, that is towards the end of September and beginning of October (see Tables XIII and XV). It should be noted also that an epidemic and epizootic began in July when the mean temperature averaged about 76° F. and the humidity was 80% (Table XIV). The largest fortnightly average number of fleas per rat was 10 in September. The smallest number, nearly 2 per rat, was noted in April when the temperature had risen to 86° F.

TABLE XV. *Statement showing the number of plague deaths in the Coimbatore municipality in fortnightly periods.*

(From Municipal Reports.)

1911 Fortnight ending	Plague deaths	1911 Fortnight ending	Plague deaths
13 May	1	9 December	22
27 „	—	23 „	21
10 June	—		
24 „	—	1912	
8 July	20	6 January	43
22 „	8	20 „	31
5 August	40	3 February	24
19 „	34	17 „	60
2 September	72	2 March	—
16 „	66	16 „	—
30 „	79	30 „	—
14 October	93	13 April	—
28 „	69	27 „	—
11 November	77	11 May	—
25 „	42		

### *Observations in Vaniambadi and Tiruppattur.*

Vaniambadi was selected as one of the centres for a year's observation partly on account of its peculiar plague history and partly as representing the condition in the central districts of the Presidency at an elevation of about 1000 feet above sea-level, at which elevation as has been pointed out (p. 719) many deaths from plague have occurred in the large municipal towns.

Vaniambadi is a large municipal town in the Tiruppattur taluq, which taluq up till December 1910 formed part of the Salem district, but in that month was transferred to North Arcot district. Vaniambadi is situated on the banks of the Palar river, the branches of which divide it into three portions. The main line of rail from Madras to Bangalore, and also from Madras to Erode and the west coast, passes through it. The elevation of Vaniambadi above sea-level is 1272 feet.

The population according to the 1901 census was 12,005, but at the time this census was taken plague was present in the town. The census taken in 1911 shows the population to be 20,340. Two-thirds of the inhabitants are Labbai Mahomedans, a people particularly difficult to deal with in sanitary matters and with whom any attempt at preventive measures which in the least approaches coercion is liable to lead to trouble. Even in the presence of plague they frequently refuse to evacuate their houses. The number of houses is now 3743 of which 374 are unoccupied.

They are of two main types :

(1) The well-built houses of the rich merchants. These are built of baked bricks covered with cement. They have good foundations, well raised plinths, flat terraced roofs and cement floors. These houses at first sight appear to be rat-proof as they seem to afford no shelter to burrowing or climbing rats; the inhabitants, however, almost invariably store bags of grain etc. in the dark corners of their houses and these piles of grain bags and accumulated rubbish furnish abundant shelter for colonies of rats, and rats can always be trapped in such houses.

(2) The poorer class of house is built of rough-baked or unbaked brick, uncemented or lightly plastered, with poor foundations. The roof is composed of several layers of the ordinary country tiles and a ceiling of bamboo lattice under the roof forms in some cases a loft in which rubbish is stored. The floors are of mud, plastered with cow-dung. Rats burrow in the walls and floors and find shelter in roofs and lofts. A certain number of the houses have thatched roofs.

The first outbreak of plague in Vaniambadi occurred in the beginning of January 1901 and the epidemic continued till April of the same year, 765 deaths from the disease having occurred in the meanwhile. No imported cases of the disease preceded the outbreak and its origin is obscure. Infection probably came from Bangalore or Oorigam in Mysore. Plague reappeared in January 1902 and continued until the end of 1903 when the town was flooded by the rising of Palar river and coincidentally therewith the epidemic came to an end. During this second epidemic 2322 deaths were recorded. As in the case of the first epidemic it was not preceded by a recognised imported case of the disease, but grain from Mysore State was suspected to be the source of infection. From April 1903 up till the time our work was commenced in Vaniambadi the town remained free from plague. Observations were commenced in Vaniambadi on 6th June 1911, a

## 734 *Epidemiological Observations in Madras Presidency*

laboratory being established under canvas. 150 traps were set daily from that date onwards and the rat and flea prevalence are given in the accompanying tables and charts (Tables XVI and XVII and Chart 29).

TABLE XVI. *Rat and flea prevalence. Fortnightly average. Vaniambadi.*

1911 Fortnight ending	Rats per 100 traps	Average fleas per rat	1911 Fortnight ending	Rats per 100 traps	Average fleas per rat
10 June	10.3	5.4	28 October	6.6	5.4
24 „	11.4	5.2	11 November	14.4	6.6
8 July	9.3	5.4	25 „	10.9	6.5
22 „	8.8	3.8	9 December	11.5	8.1
5 August	7.1	6.0	23 „	12.1	6.4
19 „	10.9	5.1	1912		
2 September	7.0	3.5	6 January	4.6	10.2
16 „	7.8	2.4	20 „	5.2	8.8
30 „	11.5	3.4	3 February	4.8	10.4
14 October	7.9	3.5	17 „	2.6	3.8

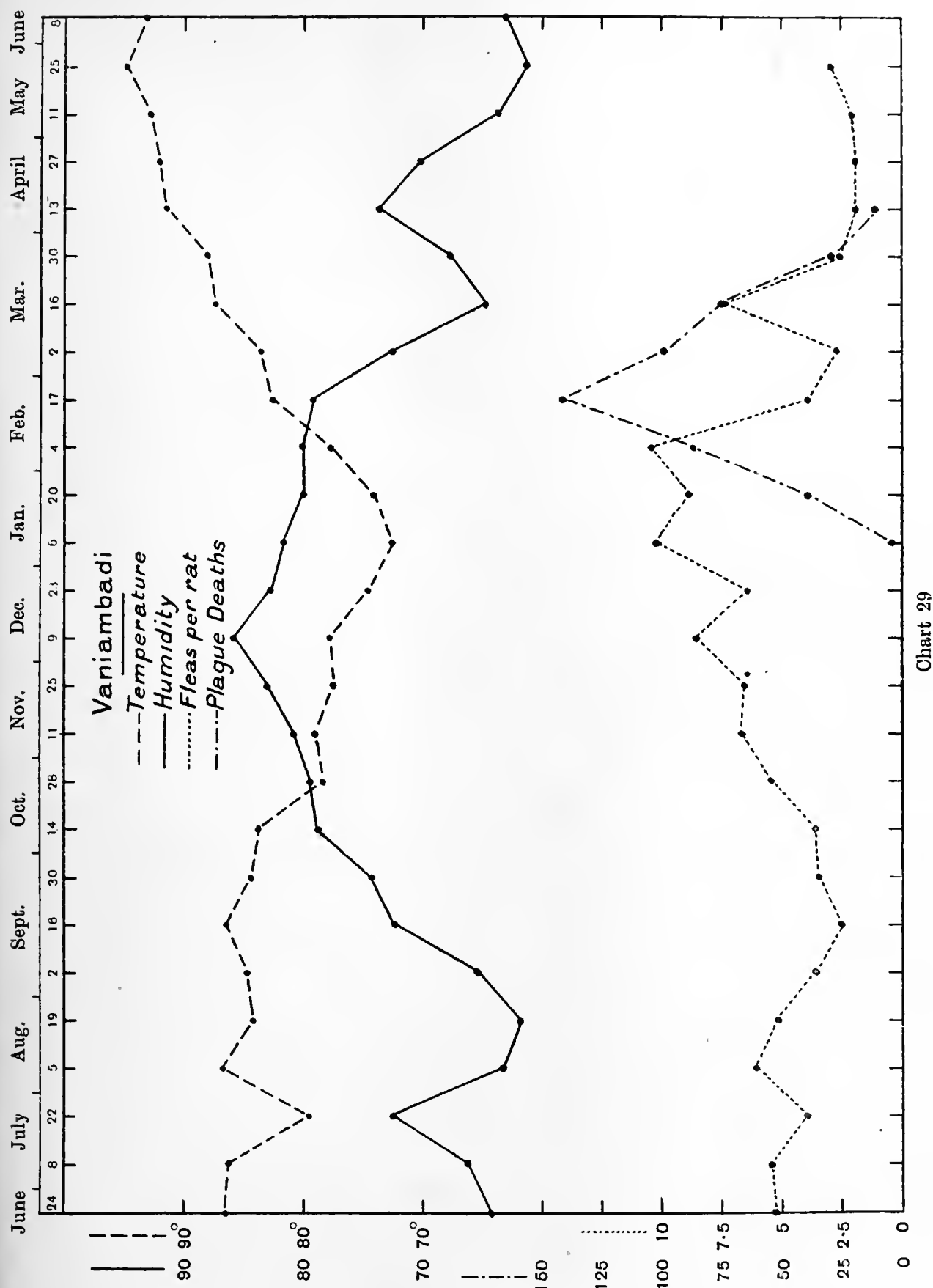
### *Tiruppattur.*

1912			1912		
2 March	8.8	2.6	27 April	7.7	1.9
16 „	9.2	7.3	11 May	4.2	2.0
30 „	5.2	2.5	25 „	2.9	2.8
13 April	6.3	1.9			

TABLE XVII. *Fortnightly mean temperature and humidity.*

1911 Fortnight ending	Tempera- ture	Humidity	1912 Fortnight ending	Tempera- ture	Humidity
24 June	86.4	64.1	6 January	72.7	81.6
8 July	86.3	67.4	20 „	74.1	80.0
22 „	79.5	72.5	3 February	77.9	80.0
5 August	86.9	63.3	17 „	82.5	79.2
19 „	84.1	61.7	2 March	83.5	72.8
2 September	84.5	65.3	16 „	87.2	64.9
16 „	86.7	72.4	30 „	88.2	67.8
30 „	84.3	74.2	13 April	91.4	73.7
14 October	83.8	78.9	27 „	91.8	70.2
28 „	78.4	79.4	11 May	92.8	63.6
11 November	79.0	80.7	25 „	94.8	61.2
25 „	77.7	83.0	8 June	93.0	62.9
9 December	77.7	85.9			
23 „	74.7	82.9			

It will be noticed from Table XVI that our observations ceased in Vaniambadi in the middle of February and that they were continued thereafter in a place called Tiruppattur. The change in the site of our



## 736 *Epidemiological Observations in Madras Presidency*

observations was forced upon us by the development of a plague epidemic in Vaniambadi in the beginning of January and by the attitude of the people towards trapping operations. With the advent of plague the people of the town ceased to cooperate with us in catching rats in their houses, many refused to take our traps, while even those who accepted them resorted to various expedients to deprive us of our captives. They believed that we were in some way responsible for the plague epidemic—seeing that up till shortly after our arrival the town had been free from the disease for as long as eight years. They also feared that, if we discovered a plague infected rat among those captured in their homes, the authorities would insist on disinfecting their houses, a proceeding which is very much resented, especially by Mahomedans who keep their women “purdah.”

Plague commenced in Vaniambadi on the 3rd January 1912, the first death occurring on that date. The epidemic continued till 11th April, 484 persons falling victims to the disease during the period. The number of deaths which occurred each fortnight is contained in the following statement.

Week ending January	6	4 deaths
„ „	20	39 „
„ February	3	87 „
„ „	17	142 „
„ March	2	99 „
„ „	16	75 „
„ „	30	28 „
„ April	13	10 „
Total		484 „

No imported case of human plague was known to have started the epidemic. Its origin is uncertain but plague was present in a village some twelve miles off at the time, from which source infection may have come. By the 10th February our catch of rats had so dwindled and the opposition of the people had become so great that it was deemed expedient to remove the laboratory to Tiruppattur. This town is situated on the main line of rail about 14 miles from Vaniambadi at an elevation of 1256 feet and enjoys a climate similar to Vaniambadi.

The population of Tiruppattur according to the 1901 census was 18,689 and according to the 1911 census was 10,470.

The lower figure in 1911 was due to the fact that the town was partly evacuated on account of plague epidemic. The Mahomedans and Hindus in Tiruppattur are about equal in number and there are

a considerable number of Brahmins. The houses are very similar to those in Vaniambadi.

Tiruppattur had previously suffered from plague. The first epidemic lasted from December 1899 to April 1900. Subsequent epidemics occurred in 1903—1904 and 1910. Only 4 or 5 cases occurred in 1912, *i.e.* up to the time the Commission finished their work in June of that year. Trapping work was commenced on 19th February and continued till 8th June.

From Table XVI it will be seen that the flea prevalence on rats in Vaniambadi attains the maximum about the beginning of January, when approximately 10 fleas per rat were recorded. The temperature and humidity (see Table XVII) were at this time 72·7° F. and 81·6 % respectively, conditions which we have repeatedly pointed out are favourable for the spread of plague and which are not generally met with in the plague-free parts of the Madras Presidency, except perhaps in the Northern Circars, which again are the most distant from plague-infected rats.

#### *Observations in Madura.*

Madura is the headquarters of the district of the same name, and is the largest city in the south of India. It is the second largest city in the Madras Presidency, with a population of 105,984 according to the 1901 census.

It is situated on the main line of the South Indian Railway, at an elevation of 447 feet above the sea-level.

After Cuddapah and Tinnevely it is one of the hottest cities in the Presidency, and it will be seen from Chart No. 13, which shows the normal temperature and humidity, that its lowest normal mean daily temperature is 77·7° F. and the temperature is below 80° F. for only seven weeks in the year. The highest normal humidity is 75·7.

Madura has never suffered from plague, and on this account was chosen to control our work in places which have had epidemic plague. The nearest place to Madura which has suffered from plague is Dindigul, a town on the South Indian Railway within two hours' journey by rail from Madura. There is a great deal of traffic between the two places, and in the year 1910, Dindigul, which suffered from plague, sent 18,702 maunds of grain and 45,964 passengers to Madura.

The house construction in Madura is very similar to that of other towns in the south of India, the commonest type of house being composed of brick walls on a low plinth, with a roof of country tiles. There

## 738 *Epidemiological Observations in Madras Presidency*

are also a large number of well-built houses with cemented walls and floors and flat terraced roofs. These houses, like the similar ones in Vaniambadi, harbour a large number of rats.

Operations were commenced in Madura on 17th April 1911 and continued till 13th April 1912. Trapping at first was started with 130 traps, the number being later increased to 250 and subsequently reduced to 200. Opposition to trapping was met with in some quarters of the town, and some small areas had to be omitted from the scope of our work, but the trapping results give a fair idea of the rat population of the town as a whole (Tables XVIII and XIX and Chart 30).

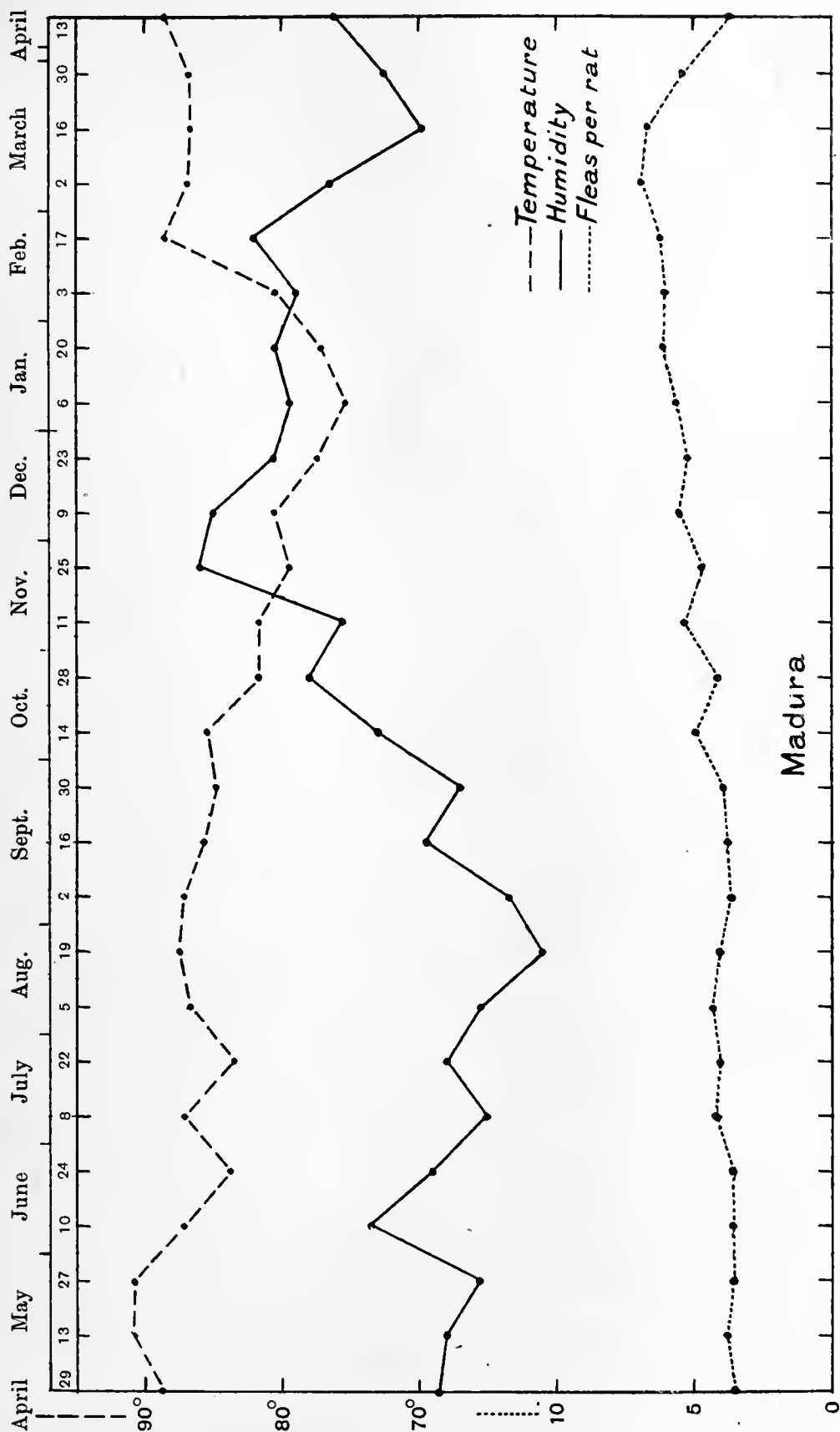
TABLE XVIII. *Rat and flea prevalence in Madura.*

1911 Fortnight ending	Rats per 100 traps	Fleas per rat	1911 Fortnight ending	Rats per 100 traps	Fleas per rat
29 April	36.6	3.5	11 November	34.7	5.2
13 May	49.6	3.7	25 „	34.1	4.7
27 „	49.6	3.6	9 December	31.0	5.5
10 June	24.7	3.6	23 „	32.4	5.1
24 „	24.7	3.6			
8 July	19.9	4.2	1912		
22 „	20.3	4.0	6 January	34.7	5.6
5 August	22.3	4.3	20 „	37.2	6.1
19 „	24.4	4.0	3 February	28.3	6.0
2 September	29.5	3.6	17 „	31.8	6.1
16 „	29.0	3.8	2 March	34.6	6.8
30 „	26.9	3.9	16 „	30.1	6.6
14 October	28.8	4.9	30 „	25.7	5.3
28 „	26.7	4.1	13 April	33.4	3.7

TABLE XIX. *Fortnightly average temperature and humidity.*

1911 Fortnight ending	Mean temp.	Mean humidity	1911 Fortnight ending	Mean temp.	Mean humidity
29 April	88.6	68.5	11 November	81.7	75.5
13 May	90.9	68.0	25 „	79.4	86.0
27 „	90.8	65.5	9 December	80.5	85.0
10 June	87.2	73.5	23 „	77.5	81.5
24 „	83.7	69.0			
8 July	87.1	65.0	1912		
22 „	83.4	68.0	6 January	75.4	79.5
5 August	86.7	65.5	20 „	77.1	80.5
19 „	87.4	61.0	3 February	80.4	79.0
2 September	87.2	63.5	17 „	88.6	82.0
16 „	85.7	69.5	2 March	86.8	76.5
30 „	84.9	67.0	16 „	86.7	69.8
14 October	85.4	73.0	30 „	86.9	72.6
28 „	81.7	78.0	13 April	88.9	76.4





Madura

Chart 30

## 740 *Epidemiological Observations in Madras Presidency*

One of the prominent features of our work in Madura was that a larger number of rats could be caught in this town than in Denkanikota, Coimbatore or Vaniambadi. No very great importance can be attached to this finding, for we must remember that the places just mentioned had or were suffering from plague and the people living in them generally believed that if infected rats were caught in their houses they would be subjected to the various inconveniences associated with the measures taken to prevent the spread of the disease. The people of Denkanikota, Coimbatore and Vaniambadi therefore assisted us less heartily in the capture of rats, adopting a variety of expedients either openly or more or less concealed, to deprive us of the rats. They have been seen for example to block up the entrance to the trap with rags or other materials, to cover over the trap with a basket or even to hang the trap on a peg out of reach of the rats till it was collected by our assistants.

Another prominent feature of our work in Madura was that the flea prevalence on the rats was never very high and remained at a fairly constant figure throughout almost the entire year—a maximum of nearly seven fleas per rat being recorded in March, while the minimum number never fell below three.

A reference will later be made to the susceptibility of the Madura rats to plague infection; it will suffice here to state that, as compared with those of Bombay and Bellary the rats of Madura were much more susceptible to plague infection.

### *Other Observations on Rat and Flea Prevalence. In Madras Presidency.*

In addition to the systematic observations in the four main centres just described, observations on a smaller scale were also made at the following places:

- (1) On the Nilgiri Hills, at Ootacamund and Coonoor.
- (2) On the West Coast, at Mangalore, Calicut and Cochin.
- (3) At scattered points in the Presidency, Bellary, Trichinopoly, Vizianagram, and Cuddalore.

At some of these places the observations were made by the trained and experienced staff of the Commission, at other places the work of counting fleas was left in the hands of a native sanitary inspector whose work was supervised at intervals.

Observations in the Nilgiri Hills.

At Ootacamund.

Two observations were made as follows :

	<i>Mus rattus</i> per 100 traps	Average fleas per <i>Mus rattus</i>
3 August to 15 August 1911	6.0	4.2
16 November to 26 November 1911	4.5	2.5

Continuous observations made by the Sanitary Inspector gave the following figures :

1911 Month	Average fleas per <i>Mus rattus</i>	1912 Month	Average fleas per <i>Mus rattus</i>
August	4.2	January	3.4
November	3.9	February	2.1
December	3.5	March	1.3
		April	0.9
		May	0.9

Of the fleas found on rats and mice :

58.6 % were *Xenopsylla cheopis*.

22.4 % were *Ceratophyllus fasciatus*.

7.9 % were *Ctenopsylla musculi*.

The remainder were *Ctenocephalus felis* and *Pulex irritans*.

At Coonoor.

The monthly flea prevalence was as follows :

1911 Month	Average fleas per <i>Mus rattus</i>	1912 Month	Average fleas per <i>Mus rattus</i>
May	4.0	January	3.4
June	4.2	February	4.9
July	3.0	March	3.7
August	3.2	April	3.1
September	3.2		
October	6.3		
November	5.0		
December	7.6		

Of the fleas on *M. rattus* :

76.9 % were *Xenopsylla cheopis*.

12.5 % were *Ceratophyllus fasciatus*.

6.9 % were *Ctenopsylla musculi*.

3.7 % were *Pygeopsylla alladinis*.

Observations in towns on the West Coast

At Mangalore.

Three observations gave the following results :

## 742 *Epidemiological Observations in Madras Presidency*

	<i>Mus rattus</i> per 100 traps	Average fleas per <i>Mus rattus</i>
29 June to 15 July 1911	2·3	4·5
10 September to 21 September 1911	2·7	4·3
20 January to 29 January 1912	2·5	5·9

### *At Calicut.*

Three observations gave the following results:

	<i>Mus rattus</i> per 100 traps	Average fleas per <i>Mus rattus</i>
21 June to 28 June 1911	24·4	4·0
20 August to 9 September 1911	13·4	3·1
7 January to 16 January 1912	15·1	4·1

The continuous count made by the Sanitary Inspector gave the following results:

1911 Month	Average fleas per <i>Mus rattus</i>	1912 Month	Average fleas per <i>Mus rattus</i>
June	4·0	January	4·3
July	4·2	February	5·3
August	3·3	March	6·0
September	3·4	April	4·6
October	3·7	May	5·0
November	4·6		
December	3·8		

### *At Cochin.*

Two observations were made as follows:

	<i>Mus rattus</i> per 100 traps	Average fleas per <i>Mus rattus</i>
5 February to 19 February 1912	7·6	2·3
11 May to 22 May 1912	3·4	1·6

A few *Mus decumanus* were found in Cochin.

### *Observations in Bellary town.*

This town was visited on four occasions for the purpose of taking a flea count.

	Average fleas per rat
4 October to 13 October 1911	5·5
11 November to 20 November 1911	5·6
20 January to 29 January 1912	7·3
13 March to 22 March 1912	2·6

*Observations in the town of Vizianagram.*

This town is situated in Vizagapatam district in the Northern Circars. It gave the highest rat prevalence of any place visited in the Presidency as judged by the figure of rats per 100 traps.

	Rats per 100 traps	Average fleas per rat
23 February to 3 March 1912	79.1	4.4

*Observations in the town of Trichinopoly.*

This town was visited once and the rat and flea prevalence was as follows :

	Rats per 100 traps	Average fleas per rat
13 October to 20 October 1911	26.5	2.5

*Observations in the town of Cuddalore.*

One observation gave the following figures :

	Rats per 100 traps	Average fleas per rat
16 October to 20 October 1911	25.3	6.1

Now we may summarise these observations by pointing out that in none of the places examined was the number of rats and fleas too few to prohibit the development of an epidemic in them. Severe epidemics have occurred in Bellary and the Nilgiri Hills, where the fleas and rats are less numerous than in places which have been free from the disease, but on the other hand the climate in Bellary and especially in the Nilgiri Hills is more favourable to the spread of plague epidemics than in the plague-free places. At Denkanikota, where the fleas are on the whole more numerous, nearly fifteen per rat, epidemics have been more severe and persistent than in Coimbatore and Vaniambadi, in which places only ten fleas per rat were found. Plague has not occurred in Madura and Cuddalore where approximately six fleas per rat were noted.

It must be remembered however that the number of fleas found on rats is dependent in great measure on the climate, as is well illustrated in the remarkable seasonal variations in the number of fleas caught on rats. The presence or absence of plague in a place depends not only on the number of fleas found on rats, although this undoubtedly is a factor of considerable importance, but also on the influence of climate on the length of period during which a flea can remain alive when

separated from its host as well as on the facilities afforded for the transport of these insects from infected to uninfected localities. It is difficult to estimate the relative value of these two latter factors in influencing the distribution of the disease; in one place the one, in another the other may be of greater importance. In the Hosur taluq of the Salem district and in the Nilgiri Hills, for example, the influence of climate appears to be of greater importance than the facilities for transport, while probably in the Bellary district and especially in the districts adjacent to it the facilities for communication in the shape of railway lines are of greater importance than climate; this, save in exceptional years, is relatively unfavourable to the spread of the disease owing to its dryness. The low temperature in the winter months however in Bellary favours the prolonged existence of the flea when separated from its host and in this respect conduces to the spread of the disease. The low-lying hot and dry plains which separate the more humid east coast districts from plague-infected places must serve as a barrier to the importation of infection by means of fleas to these relatively favourable areas for plague epidemics.

VII. *Experiments to test the susceptibility of rats from various parts of the Madras Presidency to plague infection.*

These experiments have been reported in detail in the *Journal of Hygiene*, Plague Supplement II, 1912, pp. 229—265, to which the reader is referred for an account of the technique adopted. It is necessary here merely to extract from that paper the results of the particular experiments which refer to places in Madras and which are reproduced in the following table.

TABLE XX. *Comparing the susceptibility of rats caught in the Madras Presidency with those caught in certain plague-infected towns in other parts of India.*

Experiment No.	Date	Dose	Madras	Madura	Calicut	Podanur	Palghat	Vaniambadi	Coimbatore	Bellary	Bombay	Poona	Cawnpore	Yeotgaon	Raipur
XVI	21/6/1911	1,100,000 gr.	100	100	—	—	—	100	—	—	45	—	20	—	—
XVIII	1/9/1911	12,000 bacilli 1,100,000 gr.	95	90	91	—	89	—	—	—	16	18	17	—	100
XXII	27/4/1912	14,000 bacilli 1,100,000 gr.	97	83	—	92	—	—	60	40	27	13	—	79	—

These experiments demonstrate that rats caught in certain towns in the Madras Presidency which have been practically free from plague are very susceptible to the disease. The rats caught in Bellary, however, are highly immune, while those from Coimbatore are slightly less so, but both these towns have suffered from a series of plague epidemics.

In discussing the results of these experiments in the paper referred to above we arrived at the conclusion that, during the course of a succession of severe plague epidemics in a place, a race of rats is ultimately established, the individuals of which are comparatively immune to the disease. This is probably brought about by the destruction of susceptible rats in the recurring epidemics and the survival of the more immune who are capable of transmitting their immunity to their offspring. The fact that almost all the rats caught in Madras are extremely susceptible to plague indicates, we think, that those areas where such rats are found have escaped the plague even in the earlier epochs when plague was known to have existed in other parts of India, for example in Bombay and in Delhi. This suggestion is supported by history, for we can find no record of previous outbreak of plague in southern India although a number of references to the disease in Bombay, the Punjab and United Provinces are available. If any weight can be attached to the arguments we have advanced above, they would support the view that the comparative freedom of the Madras Presidency from plague epidemics is due largely to physical causes, the climate being on the whole unfavourable to the spread of the disease.

VIII. *The measures adopted for the prevention of plague, especially those peculiar to the Madras Presidency.*

No account of the distribution of plague in the Madras Presidency would be complete without some reference to the measures adopted there for the prevention of the spread of the disease, more especially because plague has been largely confined to the districts situated on the boundaries of the Presidency which adjoin the plague-infected areas of other administrations and has shown little tendency to extend to the interior of the Presidency. The Government of Madras, despite the comparatively small death rate from plague, have been more energetic and consistent in their anti-plague measures than elsewhere and it has been maintained that the plague regulations of Madras which from

their nature can only be effectively enforced in the Presidency itself are responsible for the limitation of the disease.

The plague regulations of the Madras Presidency which were organised by Col. W. G. King, C.I.E., the late Sanitary Commissioner with the Government of Madras, differ materially from those adopted by other administrations in India in that a system of surveillance of the movement of travellers from plague-infected places is in operation. The measure was instituted immediately after plague was declared to be present in Bombay. This system has been called "The Passport System" because each traveller from a plague-infected area is required to take out a passport and to report himself at some selected office at the place of his destination daily for ten days after his arrival. The passporting however is only one of the numerous links in the chain of organisation which aims at the prevention of the spread of plague and may be regarded as "The Intelligence Branch" of that organisation. The whole system for its success as well as for its financial economy depends upon the coordination of the various agencies employed. As a result of its working the District Officers, the Sanitary Commissioner and the Government of the Presidency are kept informed by telegram followed by detailed reports of all imported cases of plague in a place and until three indigenous cases have occurred in it. All concerned are thus afforded ample time to gauge the direction of travel of epidemics and to determine what areas are immediately threatened. The areas held to be immediately threatened are at once by special order of Government "protected" by special sanitary observation staffs of predetermined proportions and nature. After a person has reported his arrival from a plague-infected place no further steps such as disinfection of his belongings are adopted, and if he does not develop plague the daily reporting for ten days concludes the measures taken. If however the first passportee develops plague, the machinery referred to above is set in motion and an endeavour is made by the prompt despatch of a trained staff to check the further progress of the disease. Those measures are utilised which have generally been recognised as useful in combating the plague, namely, evacuation, disinfection, rat destruction and inoculation.

While there can be little doubt that the excellent sanitary organisation, which is associated with the passport system and which has been briefly referred to above, may have had some effect in delaying the dissemination of plague through the Presidency, it by no means follows that the comparative immunity of the Presidency as a whole



can be attributed entirely to the sanitary measures employed. Pass-  
porting is a measure eminently valuable in dealing with those epidemic  
diseases in which the patient is a direct source of infection to the  
community, for example, smallpox, cholera or pneumonic plague, but in  
the cases of bubonic plague so commonly rat borne, and where the  
individual may carry infection whether he suffers from the disease or  
not, the only obvious advantage of passporting is that in some instances  
earlier information is obtained of a possible infection of the rat  
population.

The value of passporting as an anti-plague measure will depend  
upon:

(1) The frequency of transmission of infection by human beings  
when compared with that of other agencies.

(2) How far the probability of carrying infection is greater in the  
case of an individual who develops the disease than that of one who  
remains well.

(3) The efficiency with which the system is carried out, that is, the  
reliability of the agency employed.

With regard to (1), the evidence we have collected from various  
sources favours the view that plague-infection is commonly imported  
into fresh localities by human agency, either on the person of the  
traveller or in his effects, but, it must be remembered, that merchandise  
such as grain or fodder, in which rats abound, also constitutes an  
excellent vehicle for the conveyance of infection from the rats of one  
place to those of another.

With regard to (2), while the fact that an individual who develops  
the disease indicates that he has actually been in very intimate contact  
with infection and will therefore be the more likely to carry infection with  
him, it must be borne in mind that it has been recorded frequently  
that plague infection has been introduced into a locality by healthy  
individuals who have arrived from infected areas. The risk of carriage  
of infection by the healthy individual may be much less than by an  
infected person but the former comprise the great majority; upwards  
1000 to 1 of all persons passported being healthy.

With regard to (3), the personal observations of the working of the  
passport system by Members of the Commission during recent years  
showed that many imperfections existed. Innumerable difficulties  
arise in the practical enforcement of the regulations, more particularly  
is this the case when panic seizes the multitude as when an epidemic  
of plague breaks out in a place for the first time.

The fact that plague has been more or less confined to the districts contiguous to the infected areas of Mysore, Bombay and the Nizam's Dominions (see Map 1), has been regarded as evidence showing the effect of the sanitary measures employed. These measures may have played some part, but, as we have pointed out, the districts in question show physical and climatological peculiarities different from the rest of the Presidency. This fact is significant, for upon similar changes in temperature and humidity the seasonal distribution of plague in other parts of India has been shown to depend.

It has been our object in the previous pages of this paper to show that the physical geography of the Presidency influences its climate in such a way as from the knowledge at our disposal might be expected to limit the distribution of plague to those areas immediately surrounding the Mysore plateau. The extraordinary susceptibility of the Madras rats to plague, in conjunction with the absence of any historical record of the disease in this part of India, suggests that the present immunity of the province is not exceptional and that the Madras Presidency has escaped plague not altogether because of the measures which have been adopted to prevent its spread during the present pandemic, but largely owing to climatic or other peculiarities.

The evidence that in India bubonic plague is determined by the simultaneous occurrence of the disease amongst rats, and that the rat flea is the agent whereby the virus is transported from rat to man, is overwhelming. The work already accomplished by the Commission has also shown that the climate of the greater part of Madras Presidency is unfavourable to the continued existence of fleas apart from their host, so that the conclusion that this peculiarity is climate, is consistent with the facts.

Plague dies out sooner or later in any town or village. In the absence of large cities it would seem that in order to maintain over a number of years the existence of plague in a district the disease must be constantly transferred from one place to another. If climatic or other conditions prevent or limit this transference of infection from one place to another, the district as a whole never can become severely infected and the disease dies out in time. In the warmer parts of the Madras Presidency the climate is, in our opinion, such as not only to hinder the primary introduction of the infection but also to prevent its diffusion from place to place.

SUMMARY.

1. From 1898 to 1910, 90% of the plague deaths in the Madras Presidency have occurred in certain districts which immediately adjoin infected areas in the Bombay Presidency and Mysore State. The Bellary and the Nilgiri districts as well as the Hosur taluq in Salem district and the Kollegal taluq in the Coimbatore district have been most severely and persistently infected.

2. In certain years, especially when the temperature has been lower and the humidity higher than normal, plague has extended to places immediately adjacent to the infected areas of the Madras Presidency mentioned above and serious epidemics of plague have developed especially in certain large municipal towns while the disease in the surrounding villages has generally been mild.

3. Although plague has invaded the Madras Presidency it has shown little tendency to flourish in places beyond the limits indicated above. These limits had been attained by the year 1904. Such districts as have been affected for the first time after 1904 have returned comparatively few deaths from this disease.

4. While the districts which have suffered most from plague have the characteristic in common that they are in the closest proximity to infected areas outside the Presidency, they also resemble each other in being the most elevated and coolest parts of the Presidency.

5. A low-lying comparatively hot and dry plain separates the areas at present infected from the more humid and cooler coastal regions especially in the north of the Presidency. Plague has rarely occurred on the east coast<sup>1</sup> but on the west coast the seaport towns of Mangalore and Calicut which have intimate trade relations with Bombay have suffered from the disease.

6. In seeking for an explanation of the limited distribution of plague in the Madras Presidency it is difficult to evaluate the influence of the various factors which favour or prevent the spread of infection. For any given place, a number of circumstances, some more, others less favourable to the development of the disease, are at work together. Thus, while the severely infected areas of the Madras Presidency are situated in

<sup>1</sup> It is interesting to note that the small outbreak which occurred on the outskirts of the Madras City in 1905 and of which the origin was not definitely traced but in which there was a suspicion that infection was imported from Rangoon by sea, was followed years later (in December 1913) by a similar outbreak in another coast town—Negapatam, and in this case infection was also supposed to have come from Rangoon.

close proximity to similarly infected areas in charge of other administrations, being for this reason more open to infection, these same areas enjoy a climate which approximates to that which, from a study of plague in other parts of India, we have come to regard as favourable for plague. It appears that the proximity to infected areas and the facilities for communication with them seem to have been more important than its climate in determining the plague incidence in the Bellary district. This is supported by the fact that the Kurnool district with a very similar climate but more distant from infected centres and with less rapid and efficient means of communication has suffered but little from plague. On the other hand plague has been severe in the Nilgiris where the population is scattered and the means of communication are indifferently but where the climate is very favourable for the diffusion of the disease.

7. An examination of rats and fleas found in places selected in (a) the severely infected area, (b) the moderately infected area, and (c) in the plague-free areas of the Presidency has shown that in none of them, in the light of the Commission's experience elsewhere, was the number of rats and fleas too small to prohibit the development of an epidemic in them. Severe epidemics have occurred in the Bellary district and the Nilgiris where rats and fleas are less numerous than in places which have escaped infection. In the Bellary and Nilgiri districts other factors favourable for the development of plague epidemics, such as facilities for communication with infected districts, or a suitable climate, may be supposed to have made up for the deficiency in rats and fleas. Yet in Hosur taluq, for example, where fleas are numerous on the rats (nearly 15 per rat at the height of the plague season), epidemics have on the whole been more severe and persistent than in Coimbatore and Vaniambadi at which places 10 fleas per rat was the maximum number found. In Madura and Cuddalore, where approximately the maximum average number of fleas was only 6 per rat, plague has not occurred. We must remember however that the number of fleas found on rats depends to a great extent on the climate, as is well exemplified in the marked seasonal variation in the number of fleas found on rats in other places.

8. Experiments have shown that rats caught in places in Madras which have been free from plague epidemics are very susceptible to plague infection. This is a factor favourable to the development of plague. The existence of such susceptible rats at the present time in places in India, in view of the fact that plague has now been present in

the country for more than 17 years, indicates that conditions exist in those places which have hindered the successful implanting of infection in them. What these conditions are it is difficult to determine, but we are inclined to attribute this comparative immunity to the warm climate obtaining over the greater part of the Presidency.

9. As plague is carried from place to place in the bodies of infected fleas, any local conditions affecting the survival of these insects apart from a host are of importance in determining the liability of the neighbourhood to plague. Even when infected rats are transported from place to place, there is nevertheless an interval when infected fleas are separated from their host before they may find access to man, during which they are subject to these conditions.

When separated from their host, rat fleas speedily succumb to the combined effects of a high temperature and drying.

Unfavourable conditions of this kind are found in the hot and dry plains which separate the cooler and moister sea-coast districts from the existing areas of infection in the Madras Presidency. According to our thinking, this area breaks the chain of communication between the infected area on the west and the cooler regions of the east coast. At the same time facilities for the importation of infected rats to the coastal districts by way of the sea are poor, for no very satisfactory harbour exists on the east coast of the Madras Presidency where ships can discharge their cargoes directly upon the wharves.

10. We therefore arrive at the conclusion that the physical features and climate of the Madras Presidency have an important influence in limiting the distribution of plague in it.

## LXXIII. FURTHER EXPERIMENTS ON VACCINATION AGAINST A BODY-STRAIN OF PLAGUE.

BY SYDNEY ROWLAND, M.A., M.R.C.S.,  
*Of the Lister Institute, London.*

I FIRST recognised that a difference as regards antigenic properties existed between a body-strain and a virulent agar strain of plague from the fact that in order to vaccinate against the former it was necessary to introduce serum proteins as a source of nutriment for the bacilli which were destined for the preparation of the antigen.

In the report (this *Journal*, Plague Supplement III, Jan. 14, 1914, p. 409), "On the influence of cultivation in serum-containing media upon the virulence and immunising properties of the Plague Bacillus," the method of adding heated horse serum to the nutrient agar upon which the organisms from which the antigen was extracted were grown, was shown to yield a vaccine which in doses of 0.02 mg. protected 75 % of rats against a virulent body-strain. It has been attempted to improve upon these figures by varying the method of preparation. The procedure consisted in (1) varying the source (strain) of the organisms, (2) varying the medium upon which these were grown.

The following four methods were employed:

Method 1. The source of the organisms was a virulent agar strain propagated in the laboratory during several years. This was grown in 10 % serum broth for 24 hours. This culture was used to inoculate Roux flasks containing nutrient agar, the surface of which had been flooded with horse serum, which had been heated for half an hour at 55° C.

Method 2. The source of the organisms was as in Method 1. They were grown as before in serum broth. The surface of the agar was in this case, however, flooded with a heated horse serum extract of ox spleen.

Method 3. The source of the organisms was the spleen of a rat dead of plague. They were grown in fresh horse serum and inoculated on to the surface of agar flooded with a fresh horse serum extract of ox spleen.

Method 4. In this method the source of the organisms was, as in the last method, the spleen of a rat dead of plague. These were grown in fresh rat serum and inoculated on to the surface of agar flooded with fresh rat serum extract of fresh rat spleen.

Four antigens were prepared from the organisms grown in these various media, which will be seen to form a graduated series in which the conditions obtaining in the body of a living rat are gradually approached.

The growth was in each case killed by chloroform and swept off in salt solution. From the thick emulsion of organisms thus obtained a paste was thrown down in the centrifuge. This paste was then mixed with twice its weight of anhydrous sulphate of soda. An appropriate amount of water was added to the dry powder resulting, and the sulphate of soda removed by filtration in the form of a saturated solution at 37° C. The bodies of the bacilli remaining on the filter were extracted in distilled water.

The vaccinating power of the four antigens was then tested against a body-strain of plague obtained from the spleen of a rat dead of plague. A dose of three millions of this strain killed 90 % of uninoculated rats. The original source of this strain was a virulent agar strain which had been passed through rats twice.

The results of this experiment are shown in the following table :

Antigen No.	Strain	Culture	Agar flooded with	No. of Rats inoculated	Percentage which survived the inoculation
1	Agar	Serum broth	Heated horse serum	47	57
2	Agar	Serum broth	Serum spleen extract	39	74
3	Spleen	Fresh horse serum	Serum spleen extract	41	75
4	Spleen	Fresh rat serum	Fresh rat serum spleen extract	43	84

From these results I conclude that as the conditions in the culture medium that is used for the propagation of the organisms that are to afford an antigen approach those that obtain in the body of the living animal, so the efficiency of the antigen against a body-strain of plague is increased.

#### LXXIV. IMMUNISATION BY PSEUDOTUBERCLE.

By SYDNEY ROWLAND, M.A., M.R.C.S.,

*Of the Lister Institute, London.*

IN this *Journal* (Vol. XII. Dec. 1912, p. 350) I concluded from the results of experiments that it was possible to immunise the guinea-pig against plague by means of killed cultures of the pseudotubercle bacillus, but that it was very difficult to immunise the rat by the same method. Since the publication of these results I have discovered the important distinction as regards antigenic properties between a culture of the plague bacillus propagated on broth peptone media and on serum or in the body, respectively.

In view of this new knowledge it became necessary to repeat these earlier experiments, using a serum strain of the plague bacillus to test the immunity conferred by the pseudotubercle bacillus.

For the purpose of the tests which follow, a culture was employed that for six months had been grown continuously in the body of the living rat. When required for testing it was isolated on fresh blood agar and grown in serum broth. A dose of three million such organisms constituted the test dose.

The vaccines were prepared in the same manner as before, the pseudotubercle bacillus being grown on agar, killed by chloroform and suspended in salt solution. The vaccinating dose was ten thousand million (approximately the number present on a 24 hours' agar slope), which was the dose of organisms previously employed.

With this vaccine 26 rats were inoculated. One week after the vaccination the rats received the standard dose of living virulent plague (body-strain).

Six rats survived the test.

Similarly 28 guinea-pigs were vaccinated and tested for immunity in exactly the same way.

Four guinea-pigs survived the test.



This result is in striking contrast to that previously obtained in which no difficulty was experienced in immunising guinea-pigs against a virulent agar strain of plague.

The fundamental difference which exists between the agar strain and the serum (body) strain is emphasised in these experiments.

Similar series of rats and guinea-pigs were vaccinated, using the nucleoprotein extract as in the former series of experiments. In these series also no immunity followed the treatment.

The original statement as to the possibility of vaccinating against virulent plague by means of the pseudotubercle bacillus must now be modified to the following:

It is possible to immunise guinea-pigs against a virulent strain of plague propagated upon broth peptone media, by means of the pseudotubercle bacillus, but not possible to immunise either rats or guinea-pigs against the same strain of plague taken from the body and grown in serum-containing media, either by the whole bacillus or by means of the nucleoprotein extract obtained from it.

## LXXV. IMMUNISATION BY LIVING AVIRULENT CULTURES (STRONG).

By SYDNEY ROWLAND, M.A., M.R.C.S.,

*Of the Lister Institute, London.*

IMMUNISATION by living avirulent cultures has been advocated and actually carried out by Strong (*Philippine Journal of Science*, Vol. II. No. 3, June 1907).

By his courtesy I have had the opportunity of testing the immunising power of his culture on white rats. It proved quite avirulent both to guinea-pigs and rats, and all attempts to raise its virulence failed. Strong found that he was able to immunise guinea-pigs by the previous subcutaneous inoculation of an agar slope of his organism.

The term agar slope as a quantitative expression for a dose of bacilli is rather a vague one. From some estimations of the number of organisms present on an agar slope of average growth intensity, I adopted the number 10,000,000,000 as roughly expressing the number of organisms present.

A number of Roux flasks were inoculated with Strong's culture and the growth swept off in salt solution. The number of organisms present in the emulsion was determined by the direct counting method described in this *Journal* (Vol. XII. 1912, p. 362).

Twenty rats and twenty guinea-pigs were then inoculated subcutaneously with such a quantity of the emulsion as contained 10,000 million organisms.

A week later all the animals received a test dose of three million living virulent plague organisms from a passed culture that killed 90% of normal rats inoculated with this dose.

Of the 20 guinea-pigs 13 died of plague and of the 20 rats 9 died of plague, a survival percentage of 35 in the case of the guinea-pigs and 52 in the case of the rats.

It has been previously shown in these reports that successfully to vaccinate against a body-strain the antigen employed must be prepared

from organisms grown as far as possible under conditions obtaining in the body. (This *Journal*, Plague Supplement III, Jan. 1914, p. 408.) The experiment was accordingly made of growing Strong's avirulent culture in a serum medium and again testing its immunising power. A culture was prepared in 10% serum broth, which was then flooded on to the surface of agar. Twenty-seven rats were vaccinated with 200 million of the resulting living organisms. A week later their immunity was tested with the standard dose of virulent plague. Eight rats died of plague as the result of the test, the remaining 19 surviving, a survival percentage of 73. This is a higher survival rate than that obtained from the previous series, notwithstanding that in the previous series the dose of organisms was 50 times as great.

It is concluded that the addition of serum proteins to the culture medium had materially enhanced the protective power of the culture, a result that is in complete accord with my previous experience.

Further experiments on this point are in progress.

*The nucleoprotein obtained from Strong's culture.*

From the same strain of organisms as that used in preparing the vaccine for the last experiment (serum broth strain) a series of Roux flasks containing nutrient agar flooded with heated horse serum was inoculated. The growth obtained was used in the preparation of a nucleoprotein extract (*v. supra*).

The toxicity of this extract was, for white rats, but slightly lower than that obtained from the standard virulent strain when grown under the same conditions.

Strong strain nucleoprotein		Virulent strain nucleoprotein	
Dose	Rat	Dose	Rat
0.5 mg.	1 died 2 days	0.5 mg.	1 died 1 day
	2 „ 3 „		2 „ 1 „
0.25 mg.	3 „ 2 „	0.25 mg.	3 „ 1 „
	4 „ 3 „		4 „ 1 „
		0.1 mg.	5 „ 1 „
			6 „ 2 days

The immunising power of this extract was also determined, 30 rats receiving 0.02 mg. as a vaccinating dose. Five days later all were tested with the standard virulent culture, three million organisms being given as in the other series; 18 rats died of plague, 12 or 40% only surviving.

This result is of interest as it indicated the amount of loss in immunising value that the nucleoprotein suffers during the process of

extraction. Roughly 0.02 mg. of nucleoprotein is contained in 200 million organisms. The two series of rats therefore received the same dose of nucleoprotein. (It will be remembered that the fact that the immunising power of the plague bacillus resided in its contained nucleoprotein was established in the first of these reports, this *Journal*, Vol. IX. No. 3, Nov. 1910.) A comparison of the survival percentages in the two series of rats 73%—40% affords an index of the deterioration of the antigen due to the process of extraction.

On the other hand the rats that received the whole organism, one and all suffered from abscesses at the site of inoculation, whereas the local reaction in the case of the rats that received the nucleoprotein extract was absent. This series of rats would easily have absorbed twice the dose of the extract, in which case it is reasonable to suppose the resulting immunity would have been correspondingly higher.

Strong found (*loc. cit.*) that his culture would immunise guinea-pigs. As we have seen, the survival rate when a dose of ten thousand million organisms (one agar slope) was given, was 35%. The survival rate found by Strong was considerably higher than this, averaging 75%. This suggests that the test culture used by him was not as virulent as the test culture employed by me.

Some further experiments were made with the living organism and with the nucleoprotein extract obtained from a growth in serum containing media.

Twenty-seven guinea-pigs received 200 million living organisms each as a vaccinating dose. The organisms were grown on agar. When tested in the same manner as before 24 died of the disease. In another series 29 guinea-pigs received 0.02 mg. of the nucleoprotein extract, of this series 27 died of plague when submitted to the standard test.

From these experiments I conclude that:

1. The immunity to the standard virulent culture obtained as a sequel to vaccination with living avirulent organisms grown on agar is less than that obtained after vaccination with the antigen prepared from the same virulent culture.

2. The immunity conferred on guinea-pigs is small but distinct. This is the only means available for producing any immunity in guinea-pigs.

## LXXVI. THE INFLUENCE OF RACE ON THE EFFICIENCY OF THE ANTIGEN.

By SYDNEY ROWLAND, M.A., M.R.C.S.,

*Of the Lister Institute, London.*

A FURTHER series of rats immunised with 200 million living avirulent organisms (Strong) presents some interesting comparisons with the series already mentioned. (See this *Journal*, p. 756.)

This series was tested not with the standard virulent body-strain but with a culture received from Dr Castellani, who isolated it from a case of plague during the recent small epidemic in Ceylon (*British Medical Journal* (1914), No. 2779, p. 752). This culture was not very virulent, killing only 50% of the controls.

Of 42 rats immunised with the avirulent culture 25 or 59% survived the test inoculation.

The controls showed a survival rate of 50% so that the degree of immunity conferred appears to have been trifling, and the result suggests that the degree of immunity produced by the 'Strong' antigen against the laboratory strain is greater than against the Ceylon strain. The laboratory strain was more virulent than the Ceylon strain (as shown by tests) so that I must conclude that the two strains differ as regards their antigens. If this is true it presents another complication in preventive inoculation against plague, for satisfactory results might only follow vaccination with the particular strain concerned in any particular epidemic.

It is possible that this phenomenon may account for the poor results obtained by the Dutch authorities with Haffkine's prophylactic in Java. (*Mededeelingen van den Burgerlijken Geneeskundigen Dienst in Nederlandsch-Indië* (1912), pp. 140—148.)

To further elucidate this point two series of rats were vaccinated. Series I with the antigen (nucleoprotein extract) obtained from the Ceylon strain, Series II with the antigen obtained from the laboratory virulent strain.

Of 49 rats vaccinated with the 'Ceylon' antigen 36% survived the test by the virulent laboratory strain, whilst of 47 rats vaccinated with the virulent laboratory strain antigen and tested with the virulent laboratory strain no less than 60% survived.

It would appear then that the antigens derived from the Ceylon strain are not entirely identical with those derived from the laboratory virulent strain and that cross immunisation is not complete.

LXXVII. ON THE FAILURE TO VACCINATE AGAINST  
A VIRULENT BODY-STRAIN EVEN WITH AN ANTIGEN  
PREPARED AS FAR AS POSSIBLE UNDER BODY  
CONDITIONS.

By SYDNEY ROWLAND, M.A., M.R.C.S.,  
*Of the Lister Institute, London.*

As experience with vaccinating experimental animals (rats) in the laboratory has accumulated so the difficulties attending the work increase. One of the great difficulties I have experienced recently is the sudden and unexpected rise in virulence of the test culture. This culture has now been kept going in the bodies of living rats for nearly a year. Originally derived from a virulent agar strain it has been passed and repassed continuously. On the death of a rat the spleen organisms are isolated on agar, the surface of which is smeared with fresh heart blood. From the culture thus obtained, which is almost invariably a pure growth, a serum broth culture is made. Three million organisms from this growth have now for some time constituted the standard test for immunity. Such a dose kills 90 % of unprotected rats in from three to four days. Recently, however, the virulence of this culture has risen to a still higher degree and at present it will kill ten rats out of ten in two days. The virulence of this culture is apparent at the post-mortem examination of the animals.

The extent to which the infection pervades the animal is astonishing. The local reaction extends up the entire side of the rat, the pelvic glands and spleen even after this short period are a closely packed mass of bacilli. After a somewhat extensive acquaintance with the appearance of rats dead of plague, in the course of which I have examined many thousands of animals, I have never seen, even during the height of a Bombay epidemic, animals so severely infected. Advantage was taken of this rise in virulence of the test culture to determine the immunity conferred against it by an antigen prepared from it.

For this purpose two antigens were prepared.

Antigen No. I was prepared from a growth of the organisms on nutrient agar flooded with fresh rat serum, antigen No. II was prepared in the same manner with the exception that an intermediate culture in fresh rat serum was interposed.

With the first antigen 44 rats were inoculated. The dose was 0.02 mg. Of these 44 rats 38 succumbed to infection when tested five days later with three million organisms.

With the second antigen 49 rats were inoculated, using the same 0.02 mg. dose. Of these 34 died of plague when tested five days after the inoculation of the antigen.

Now these results are in striking contrast to those detailed in a previous Report (see this *Journal*, p. 753), where it was shown that a considerable immunity followed the inoculation of the same dose of an antigen prepared from the same stock of organisms after three passages through rats, when tested against a strain of corresponding virulence.

In practice strains of organisms of every possible degree of virulence will assuredly be met with, and should a strain corresponding to the one with which the above experiments were made be prevalent in a local epidemic, complete failure of any protective inoculation at present known will occur.

LXXVIII. THE PROTECTIVE AND CURATIVE VALUE,  
AGAINST INFECTION WITH A SERUM RACE OF  
PLAGUE, OF THE SERUM OF A HORSE IMMUNISED  
WITH NUCLEOPROTEIN EXTRACTED FROM A STRAIN  
OF PLAGUE BACILLI PROPAGATED ON SERUM  
PROTEIN.

BY SYDNEY ROWLAND, M.A., M.R.C.S.,

*Of the Lister Institute, London.*

I HAVE shown in a previous report (this *Journal*, Vol. XI. Dec. 1911, pp. 11 *et seq.*) that a protective and curative serum for rats can be prepared against a broth (laboratory) strain of plague, by means of immunising horses against a toxic nucleoprotein derived from the bodies of the same strain of plague bacilli, also propagated upon broth. The administration of such a serum in doses of 1·0 c.c. subcutaneously, completely protected a series of rats, which had received, subsequent to inoculation, a standard test lethal dose of broth organisms. The serum, moreover, exercised a curative action in the case of another series of rats, when administered in 0·5 c.c. doses, 6—30 hours after inoculation with the usual test dose, reducing the mortality from 80 to 18%/. When, however, immune serum prepared in the above manner was tried in practice in Bombay (this *Journal*, *ibid.*, pp. 326 *et seq.*) in cases of human pest, the results obtained were very discouraging and failed to show any effect whatever in influencing the course of the disease.

It was suggested to me by Dr. Martin, of the Lister Institute, that the explanation of this seeming paradox lay in the fact that the serum that gave such good results on rats in the laboratory failed in Bombay because the strain of organisms with which the serum was prepared and against which it was tested was a laboratory or broth strain. Acting on this suggestion it became of interest to ascertain whether a serum could be prepared using as a source of the immunising toxin a body-strain of organisms and using the same strain as a test of the efficiency of the serum.

The horse "Wealdstone," that was used for the preparation of the first batch of serum, was accordingly taken in hand.



## Reports on Plague Investigations in India 763

A bleeding before the immunisation was commenced was tested both against a body-strain and against a broth strain of plague with the results shown in the table :

<i>Broth race 3,000,000 organisms</i>			
Dose serum 24 hours before test			Dose serum 24 hours after test
1 c.c.			1 c.c.
10 rats, 3 died pest			10 rats, 5 died pest
0·1 c.c.	[Controls 10 rats,		0·1 c.c.
10 rats, 7 died pest	8 died pest]		10 rats, 4 died pest
<i>Serum race 3,000,000 organisms</i>			
1 c.c.			1 c.c.
10 rats, 5 died pest			10 rats, 10 died pest
0·1 c.c.	[Controls 10 rats,		0·1 c.c.
10 rats, 10 died pest	10 died pest]		10 rats, 10 died pest

This experiment shows that, as had been suggested, the serum of the horse Wealdstone (which was the serum that was sent to Bombay for Clinical experience) was still operative against a broth strain of plague but absolutely without effect against a body-strain of plague. If the test above given be compared with that given in the previous report (this *Journal*, *loc. cit.*) it will be seen that the serum was of the same order of potency as on the occasion of the last test.

These results also emphasise the difference between a serum race and a broth race, for whereas 0·1 c.c. of the serum was followed by the survival of six out of ten rats when given twenty-four hours after the test dose of broth organisms, the same quantity of serum was without effect against the same number of serum organisms (body-strain) which had been inoculated into ten rats the same time before the inoculation of the serum.

The immunisation of the horse was then proceeded with.

The material used for the immunisation was a toxic nucleoprotein extract prepared from a body-strain of plague grown in horse serum on the surface of agar in Roux flasks. (For method *vide* this *Journal*, Vol. x. Nov. 1910, pp: 536 *et seq.*)

The horse received, intravenously, doses of this extract as follows :

16—2—14	10 milligrammes
18—2—14	30        „
23—2—14	10        „
27—2—14	50        „
4—3—14	200       „
10—3—14	400       „
17—3—14	800       „
28—3—14	Bled 1 litre.

The immediate effect on the horse after each dose was, especially after the larger doses, prostrating. This soon passed off and as a rule the horse was in his usual form in the course of two days.

The test of the bleeding taken on 28—3—14 is given in the table. This test was against the same body-strain as that used for the previous test. During the time the immunisation of the horse lasted, the strain was passed continuously through rats, being isolated at intervals on fresh rat blood agar.

The number of plague organisms used for all these tests was three millions.

Test of bleeding 28—3—14 against <i>serum race</i> , 3,000,000 organisms		
Dose serum 24 hours before		Dose serum 24 hours after
1 c.c.		1 c.c.
10 rats, 4 died pest		10 rats, 3 died pest
0.1 c.c.	[Controls 10 rats, 10 died pest]	0.1 c.c.
10 rats, 6 died pest		10 rats, 5 died pest
0.01 c.c.		0.01 c.c.
10 rats, 8 died pest		10 rats, 10 died pest

The effect of the immunisation of the horse has been to render its serum operative against a serum (body) strain of plague, against which, before the immunisation, it was inoperative.

For whereas before the immunisation, of ten rats that received 1 c.c. 24 hours after the administration of the test dose, all died of plague, after the immunisation, of ten rats that received the same dose the same time after the test inoculation, only three died of plague.

Similarly with the smaller doses of serum. After the immunisation, 0.1 c.c. of serum saved five rats out of ten that had 24 hours previously received the test dose of the body-strain. Before the immunisation this dose of serum was absolutely without effect under the same conditions, not only so but ten times this dose, 1.0 c.c., was similarly inoperative.

It is concluded that just as with a broth race it is possible to prepare a curative serum against a broth race, so with the body-strain it is possible to prepare a serum operative against a body-strain.

NOTE.—It is of interest to note that the amount of toxin that was administered to the horse intravenously on 17—3—4, viz. 800 milligrammes, was contained in the growth from about 300 Roux bottles, an amount that is quite impossible to administer if given wrapped up in the bodies of the bacilli. This is one of the advantages of using the nucleoprotein extract. Given in the form of an emulsion of bacilli this amount of toxin would be contained in about three litres of thick emulsion. To put three litres of thick emulsion into the vein of a horse would probably prove immediately fatal.

## LXXIX. ULTRAVIOLET LIGHT AS A GERMICIDE IN THE PREPARATION OF PLAGUE VACCINE.

By SYDNEY ROWLAND, M.A., M.R.C.S.,

*Of the Lister Institute, London.*

IN the course of these reports it has been shown that: (1) An efficient protection against a laboratory strain of plague is easily obtained by vaccination with a nucleoprotein extract obtained by the dehydration method (this *Journal*, Vol. x. No. 3, Nov. 1910, pp. 536 *et seq.*). (2) An immunity of a high order may be obtained against a body-strain of plague by the use of a nucleoprotein extract, provided the organisms furnishing the extract are of the same strain as those against which it is desired to vaccinate (this *Journal*, Plague Supplement III, Jan. 1914, pp. 403 *et seq.*).

The use of heat being inadvisable in the preparation of such vaccines owing to its deleterious effects on the antigen, and the preparation of the nucleoprotein extract requiring a somewhat special equipment for its successful employment, other means were sought whereby it might be possible to destroy the infectivity of a vaccine without at the same time destroying the contained antigen. Could such means be found the preparation of a vaccine might be greatly simplified. The use of a whole vaccine entails in practice certain well-known conveniences of manipulation. The desideratum being then some method of killing a culture of enhanced virulence (body-strain) without at the same time so deteriorating the antigen contained within it that it is rendered useless, the well-known bactericidal action of ultraviolet light suggested itself as a method worth investigating.

### *Experiment 1 (Orientating experiment).*

A thick emulsion of virulent bacilli obtained from the spleen of a rat dead of plague, isolated on blood agar, was prepared in salt solution. (A similar emulsion was used throughout these experiments.)

This was exposed to the light of a 5 ampere arc for one hour, using a quartz cell and a quartz collecting lens.

Ten control rats inoculated with 20 million organisms. All died of plague.

Ten control rats inoculated with the same dose that had been exposed to the same radiation employing a glass collecting lens, all died of plague.

Of ten rats inoculated with the same dose of the emulsion exposed to the radiation collected by a quartz lens, one died of plague.

This result shows that the bactericidal radiation is stopped by glass, *i.e.* that the active rays are of shorter wave length than about 330 micro mu.

#### *Experiment 2.*

The same experiment was repeated, using a quartz collector, and the time of exposure was varied from 40 minutes to five minutes. Of the entire series of rats inoculated with a dose of 20 million organisms all survived.

As short a time as five minutes is therefore sufficient to kill the plague bacillus when exposed to the radiation from a carbon arc.

#### *Experiment 3.*

The same experiment was repeated, exposure times as short as one minute being employed.

The results are shown in the following table. Six rats were in each case inoculated with 30 million organisms.

Controls	all died of plague		
1 min. exp.	4	„	„
2 „	5	„	„
3 „	4	„	„
4 „	1	„	„
5 „	1	„	„
8 „	0	„	„
10 „	0	„	„

The previous result was thus confirmed.

The survivors were then, after an interval of ten days, tested for immunity. All died of plague.

The inoculation of 30 million organisms, killed by ultraviolet light, had therefore produced no immunity.

*Experiment 4.*

The last experiment was repeated, using nickel cored carbons which afford a particularly rich ultraviolet spectrum, with the following results :

Controls	all died of plague		
1 minute	5	„	„
2 minutes	3	„	„
4 „	0	„	„
10 „	0	„	„

The survivors were then, after an interval of ten days, tested for immunity. None was evident.

There were two survivors from the six rats that had received the organisms submitted to two minutes' exposure, and two from the six rats that had been submitted to four minutes' exposure to the radiation.

*Experiment 5.*

The last two experiments were repeated, using the radiation from a quartz mercury lamp.

No. rats	Min. exposure	Fate after test
6	1	2 survived
6	2	1 „
6	4	2 „
6	10	0 „

All the rats survived the preliminary inoculation with the radiated organisms.

The conclusions to be drawn from these experiments are that: (1) Ultraviolet light kills plague bacilli with facility. (2) The killing of the organisms is accompanied by destruction of the antigen. There was still a chance, however, that by using rays of definite wave length a differential effect might be obtained, and that a wave length could be found that whilst being bactericidal was not capable of injuring the antigen.

I therefore set about to ascertain the wave length of the rays responsible for the killing.

The spectrum of the light emitted by a quartz mercury lamp projected by a quartz prism and a simple lens of 40 cm. focal length was employed.

The spectrum was projected on to the surface of an agar plate previously inoculated with a virulent strain of plague. It was found

that after half an hour's exposure no growth took place beyond the line 296 micro mu.

This experiment was repeated several times and the point established that wave lengths shorter than this were uniformly bactericidal up to the extreme limit of the spectrum that the apparatus would yield, *i.e.* up to about the line 237 micro mu. We may say then that the bactericidal region of the available spectrum extends between the lines 237 and 296.

The method of plating was controlled by a direct observational method, using a broth culture of *Bacillus enteritidis*. The spectrum was projected into the quartz substage condenser of the microscope and the preparation illuminated by a subsidiary lamp, the light from which was reflected from the last face of the quartz prism. Matters were so arranged that any desired wave length could be employed. A particular organism was selected and its movement followed with the eye. By opening a shutter controlling the ultraviolet light at the desired moment the effect of any wave length on the motility of the bacillus could be observed.

Working in this way it was found that motility was immediately arrested as soon as the region 2967 was reached and that the effect was still recognizable as far as the region 2557, after which it became so slight as to be uncertain.

This experiment, which was repeated very many times, always led to the same result.

Further observations were made on infusoria and on algae and desmids. The effect of the bactericidal rays on these organisms was often very startling. One particular infusorian employed (*sp. incert*) which contained a large number of chloroplasts, on being submitted to the effect of the rays immediately burst, scattering the chloroplasts. A diatom will under the same circumstances separate its valves and extrude its contents, an alga will contract its chlorophyll bands into a central tangled mass and many other unicellular organisms will behave in curious and unexpected ways.

So far, then, the possibility of preparing a vaccine by the help of these extraordinarily energetic rays did not appear hopeful. The violence of their action was unexpected, but it is well known that many ferments are destroyed by ultraviolet light.

The next point to be determined was the extent of the spectrum that was absorbed by the antigen, which for this purpose may be taken as the nucleoprotein extract prepared by the sulphate method. The

absorption spectrum of this substance was accordingly determined, and it was found that absorption commenced at the line 2967 and extended to the extreme ultraviolet.

Now the line 2967 is the last line that permits the bacillus to grow when exposed to the influence of the complete spectrum as we have above pointed out. It appears then that that region of the spectrum which is responsible for the bactericidal effect is precisely that region that is absorbed by the antigen. It was still barely possible that there existed wave lengths that while being absorbed by the antigen were bactericidal but not capable of injuring the antigen. This was a bare possibility, but no more.

The experiment was accordingly made of exposing an emulsion of bacilli to the entire radiation of a quartz lamp through the (query) protective action of a solution of the antigen. Thirty rats were inoculated with a vaccine prepared in this manner, the exposure varying from five to 20 minutes. Subsequent test with a virulent culture, which killed all out of ten controls, also killed the whole of the 30 inoculated rats.

It is concluded from these experiments that the bactericidal and antigen injuring regions of the spectrum are identical<sup>1</sup>.

I wish to express my profound sense of obligation to Dr C. J. Martin, Director of the Lister Institute, for his help throughout the entire work on the results of which these reports are based; also to my colleagues on the staff of the Lister Institute, most of whom have helped me in one way or another during the progress of the investigation.

<sup>1</sup> At this point Dr Rowland's investigations were interrupted by the European War.

LXXX. OBSERVATIONS ON THE LENGTH OF TIME THAT FLEAS (*CERATOPHYLLUS FASCIATUS*) CARRYING *BACILLUS PESTIS* IN THEIR ALIMENTARY CANALS ARE ABLE TO SURVIVE IN THE ABSENCE OF A HOST AND RETAIN THE POWER TO RE-INFECT WITH PLAGUE.

By A. W. BACOT, *Entomologist, Lister Institute.*

VERJBITZKI (1904) records the period for which he found fleas able to carry the bacilli and re-infect as three days.

The Commission for the Investigation of Plague in India (1906) found that in their experimental godowns, infection conveyed by fleas might take place 21 days after the flea population had had any opportunity of imbibing infected blood.

Bacot and Martin (1914) found that infected fleas which were regularly fed might live for 50 days at 10° C. to 15° C. and 23 days at 27° C. and remain infected at death.

*Methods employed in the following Experiment.*

Cages similar to those used in the Indian investigation (see Report, 1906, *Journal of Hygiene*, Vol. VI. p. 435) and also by Bacot and Martin (Report LXVII, p. 429, Plate XXIV, fig. 2, Plague Supplement, *Journal of Hygiene*, 1914), after sterilization, were prepared for the reception of mice and then stocked with 100 to 300 fleas (*Ceratophyllus fasciatus*) which had been infected by allowing them, when hungry, to feed on pest-infected mice that were in the comatose condition that immediately precedes death<sup>1</sup>. By delaying the fleas' opportunity to feed until this

<sup>1</sup> Three attempts were made to infect fleas (*Ceratophyllus fasciatus* and *Xenopsylla cheopis*), which had not fed previously, on mice that had already died of pest. In two of the three cases the fleas were placed with the mouse within an hour after its death. Subsequent dissection and the examination of stomach smears revealed neither blood nor lymph, nor was there any trace of bacilli, although in each instance the heart blood of the mouse showed a heavy septicaemia. Bugs (*Cimex lectularius*), which were tried at the same time, also gave a negative result as regards the presence of bacteria, but it is not possible to state definitely that they did not obtain any fluid at all from the bodies, owing to the fact that the gut of bugs which have been starved for considerable periods generally contains some remnants of a previous meal.



acute stage of the disease it was found possible to infect a much higher percentage than was otherwise possible. Samples of each batch were dissected and smears made from their stomachs showed on microscopic examination that from 65 % to 80 % of the sample fleas carried *B. pestis*.

After stocking the cages they were tested by placing two or three healthy mice in each. In all but two of the cases (Nos. 1 and 8) these mice died of typical pest. In the great majority of cases one or both of the glands on the groin were infected, less frequently one or both of the axillary glands were also involved, and, in very few cases, the axillary glands only were infected.

The spleen was in every case infected, usually heavily, and blood from the heart always showed some bacilli, the degree of septicaemia being generally marked.

As regards cages Nos. 1 and 8, no infection occurred within the usual period of three to five days, and the mice were removed and killed under ether. After the animals (dead or living) had been removed, the cages were covered with waxed cloth in order to check drying as far as possible, and then stored in a cool situation—the temperature varying from 35° F. to 60° F. with a mean of about 45° F. or 47° F.

When the desired period had elapsed the waxed cloth cover was removed and two healthy mice added to each cage.

The following table shows that cages Nos. 2, 3 and 8 remained infective for 29, 34 and 47 days respectively.

No. of Cage	Result of Preliminary Test	Period for which the Cages were stored without any host for the Fleas	Result
1	Mice not infected	18 days	—
2	4 mice died of pest	29 „	2 mice died of pest, 1 within 4 and 1 within 5 days
3	3 „ „	34 „	1 mouse died of pest within 3 days
4	3 „ „	35 „	—
5	3 „ „	39 „	—
6	3 „ „	45 „	—
7	3 „ „	45 „	—
8	Mice not infected	47 „	1 mouse died of pest within 24 days
9	3 mice died of pest	59 „	—
10	1 mouse died of pest	68 „	—
11	1 rat and 1 mouse died of pest	73 „	—

In cages Nos. 2 and 3 it will be noted that the fleas had an opportunity of ingesting infected blood after the initial meal which infected them, but in cage No. 8 the only chance of feeding afforded them after the infecting meal was on healthy mice, which showed no signs of infection up to the time of their removal from the cage. The infection of the mice in cages 2 and 3 must have taken place within a few hours, at most, of the animals being placed in the cages after a lapse of 29 and 34 days respectively. In cage No. 8, however, there must have been an interval of about 20 days before the mouse was infected by the fleas in the cage. The records of the preliminary tests of the cages used in these experiments show that the period between the ingestion of bacilli by the fleas and the infection of a new host may be as short as three days—more usually it is longer, seven, nine or 12 days. It is of some interest to note in this connection that a period of latency in the development of plague among rats on shipping has been noticed by Dr C. Oswald Stallybrass, who, in writing me commenting on the subject, supplies the following instances: "Two cases have been brought to my notice within a month. One, a vessel from River Plate on which a small number of recently dead rats were found about five weeks after it had left the River Plate. In the second, the ship had probably been infected not later than the 29th November, while five rats dead of plague were found in one limited portion of the ship on the 15th January—nearly seven weeks later. Two of the rats were recently dead and the other three less than ten days previously. Apart from two rats apparently killed by the dock labourers, no other dead or sick rats were found on the vessel, though 70 were destroyed by fumigation; these on examination proved to be healthy."

#### *Conclusions.*

1. Fleas (*Ceratophyllus fasciatus*) are able to carry *Bacillus pestis* for periods up to 47 days in the absence of any host and subsequently to infect a mouse.
2. That infected fleas, starved for 47 days and then placed upon a mouse, may not infect it for a further period of about 20 days.
3. There is no reason to suppose that the positive results obtained in these few experiments represent the limit of time after which infection may take place, but indicate that plague infection may persist in fleas for one or two months in cool weather and, subsequently, give rise to an epizootic.

REFERENCES.

- BACOT AND MARTIN (1914), Observations on the Mechanism of the Transmission of Plague by Fleas. Plague Supplement III, January 14, 1914, p. 429 and Plate XXIV, fig. 2.
- REPORTS ON PLAGUE INVESTIGATIONS IN INDIA (1906), *Journal of Hygiene*, Vol. vi. p. 435.
- VERJBITZKI (1904), The Part played by Insects in the Epidemiology of Plague. Thesis for M.D., St Petersburg. Republished in *Journal of Hygiene* (1908), Vol. VIII. p. 162.

LXXXI. FURTHER NOTES ON THE MECHANISM OF THE  
TRANSMISSION OF PLAGUE BY FLEAS.

By A. W. BACOT, *Entomologist, Lister Institute.*

(With Plates XXXV and XXXVI and 2 Text-figures.)

SINCE the publication of the paper dealing with this subject (Bacot and Martin, 1914) further material in the form of longitudinal serial sections of infected fleas has passed through my hands. An examination of this material confirms the conclusions already arrived at, and adds one or two fresh details to our knowledge of the subject.

Text-figures 1 and 2 show the proventriculus in section and serve to illustrate the action of the opening and closing of the valve formed by the chitinized spine-like epithelial cells. When at rest the position of the spines and shape of the organ is shown in fig. 1—the opening into the stomach being free for the passage of blood. On the contraction of the muscular bands, which are arranged like the hoops of a barrel, the



Fig. 1.

girth of the proventriculus is constricted; the organ is by the same action elongated, and the lumen of the opening into the stomach is closed against the outward passage of blood (fig. 2).

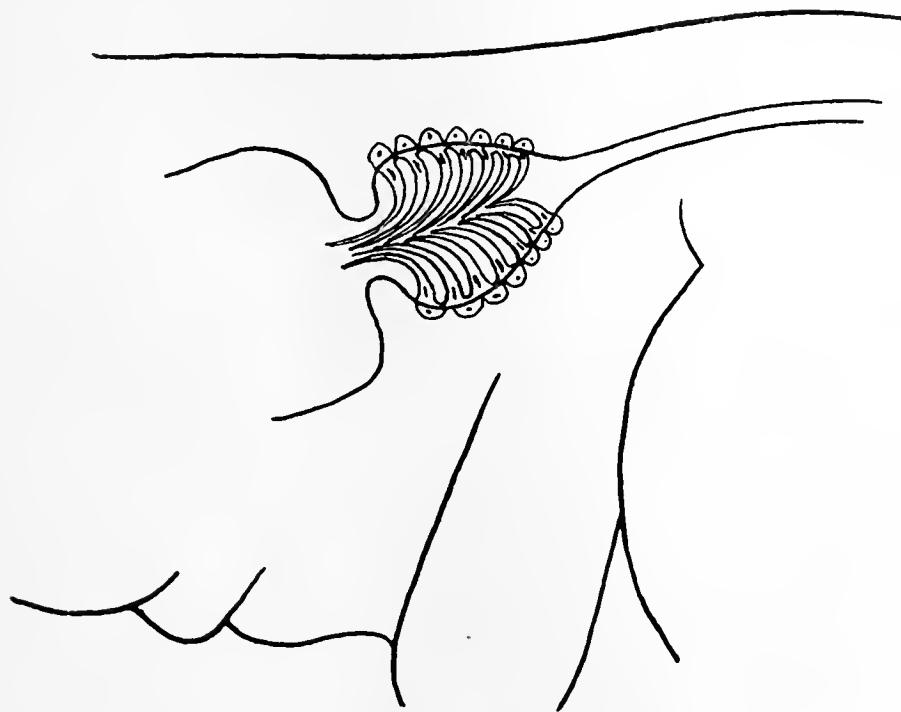


Fig. 2.

As already pointed out, it is the lodgment and growth of the bacilli among the spines of the proventriculus that is the initial stage of the blockage which during a subsequent feeding-act may lead to regurgitation of infected blood into the wound caused by the flea.

From the later sections it appears quite definite that in the first instance the block may be of a quite ephemeral nature—a second stratum forming in front of the first, both being so slight as to be depressed by the pressure of fresh blood entering through the oesophagus. When, finally, the process develops to the extreme stage shown in figure 4, Plate XXV, of the above-quoted paper, the condition is not necessarily fatal to the flea. The section of a specimen of *Ceratophyllus fuscatus*, Plate XXXV, fig. 1, shows the clearance of a passage through the centre of such a plug. The rupture of the obstructing mass does not, however, restore the lost valvular function to the proventriculus, but merely leaves a passage through which the blood can flow out of the stomach just as freely as it enters. The result, as is shown in this particular specimen, is that, after a full meal, blood, impregnated with bacteria, extends from the posterior portion of the stomach to the anterior chamber of the

pharyngeal pump. In this condition the infection of any animal on which the flea fed would seem to be more, rather than less, likely than in the case of a flea in which the proventriculus is completely blocked. With the existence of a patent proventricular valve the infected contents of the stomach may, owing to the peristaltic contraction of that organ, be regurgitated into the wound on the cessation of the suction of the pharyngeal pump.

Other sections show fresh unaltered blood in the sucking tube of blocked fleas, indicating that there is sufficient pressure in the distended oesophagus to prevent the normal clearance of the tube after a meal, which should take place owing to the action of the pump. One example shows the presence of a massive infection of such fresh blood along the course of the oesophagus to the pumping chamber, extending well down into the upper third of the sucking tube (see Plate XXXVI).

#### REFERENCE.

BACOT AND MARTIN (1914), Observations on the Mechanism of the Transmission of Plague by Fleas. *Plague Supplement III*, January 14, 1914, pp. 425—9.

#### DESCRIPTION OF PLATES.

##### PLATE XXXV.

Fig. 1 is a diagrammatic representation of a longitudinal section through the oesophagus (*oe*), proventriculus (*p*), and stomach (*s*) of a heavily infected specimen of *Ceratophyllus fasciatus*. The light shaded portion shows where fresh blood, impregnated with free individuals of *B. pestis*, is present in the specimen, the darker shading indicates the solid mass of bacteria which has so far become disintegrated at its centre, as to be ruptured by the force of the blood pumped into the oesophagus, thus allowing the passage of blood to the stomach. The action of the valve is, however, inoperative, owing to the solidity of the mass of bacteria in which the spines of the proventriculus are embedded.  $\times$  about 180.

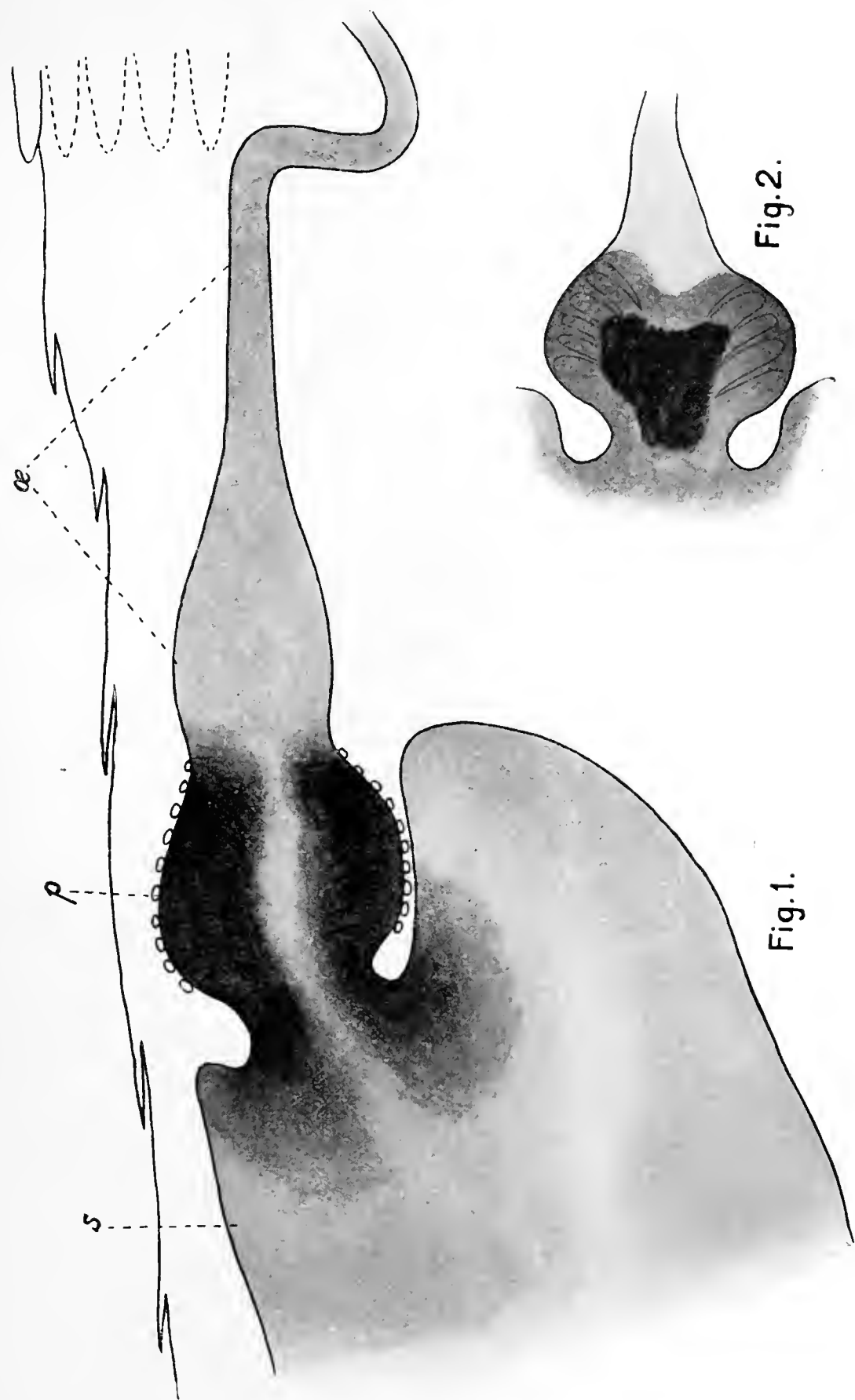
Fig. 2 shows a similar representation of a section through the dissected proventriculus and oesophagus of a specimen of *C. fasciatus*. It differs from fig. 1 in that the lumen of the valve is still obstructed by the disintegrating mass of an old plug and that the growth of bacteria surrounding this, which is of more recent growth, though yielding to the pressure of the fresh blood pumped into the oesophagus, has not yet been ruptured.  $\times$  about 180.

##### PLATE XXXVI.

Longitudinal section of head and thorax of a blocked specimen of *Ceratophyllus fasciatus*.  $\times$  about 250.

*h.*, arrangement of chitinous and soft integument in juxtaposition, forming a hinge which allows of the free ventro-dorsal movement of the sucking tube. *m.p.p.*, muscles of pharyngeal pump. *m.s.p.*, muscles of salivary pump. *oe.*, oesophagus containing a mixture of blood and bacilli. *p.*, proboscis or sucking tube. *s.d.*, salivary duct.

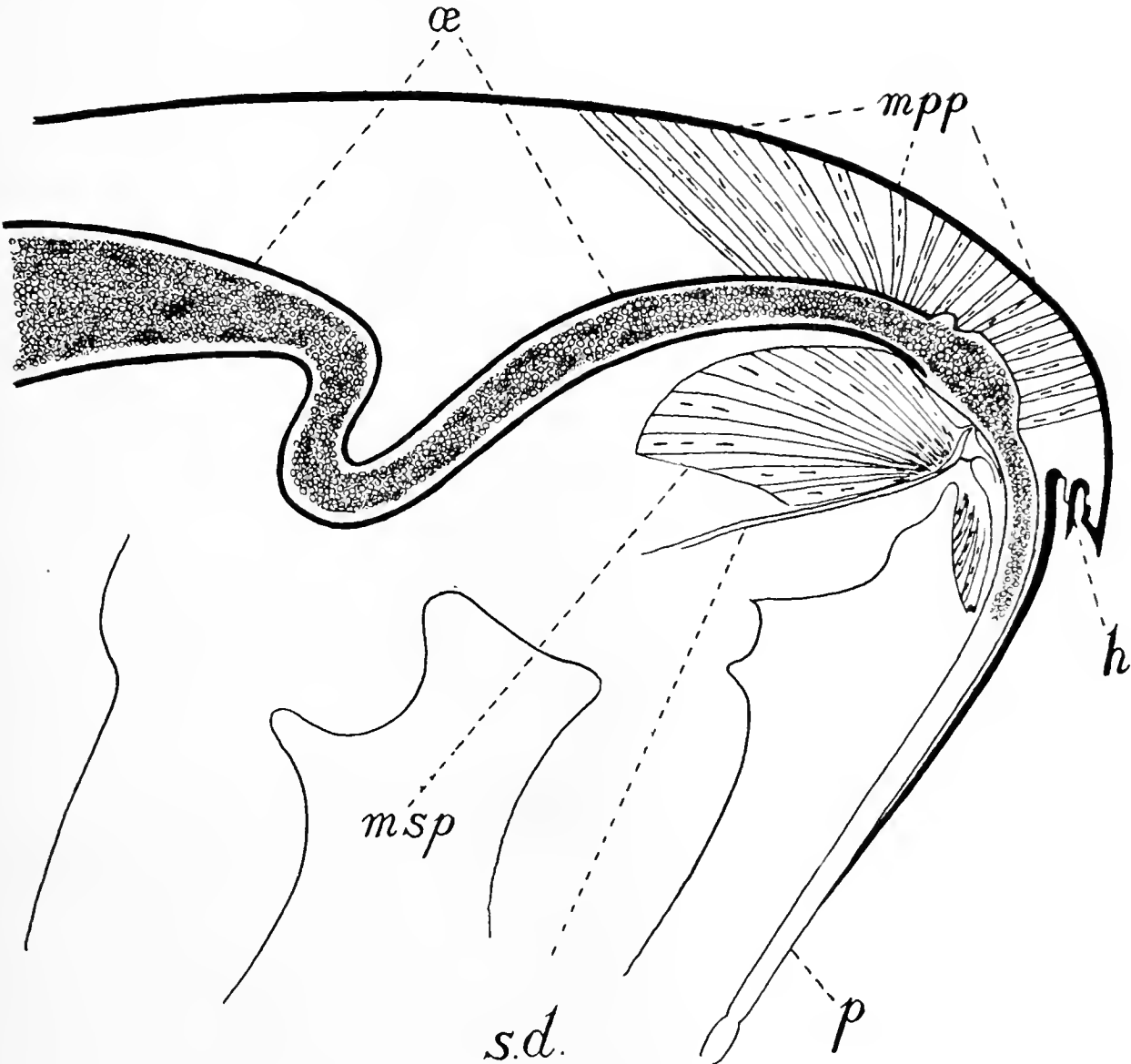
NOTE.—The bacilli are better seen with the aid of a reading lens.



Mabel Rhodes del.







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767

LXXXII. NOTES ON THE DEVELOPMENT OF *BACILLUS PESTIS* IN BUGS (*CIMEX LECTULARIUS*) AND THEIR POWER TO CONVEY INFECTION.

BY A. W. BACOT,  
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(With Plates XXXVII and XXXVIII and 1 Text-figure.)

*Introduction.*

VERJBITZKI (1904) was quite successful in his attempts to infect guinea-pigs by the bites of bugs (*Cimex lectularius*) which had previously been allowed to feed on animals that were dying of plague.

He found that allowing the insects to feed on the ear was more effective than letting them bite the leg; while, apart from situation, the virulence of the strain which killed the animals on which the bugs were fed was the crucial factor.

When the virulence was low there were no deaths, but, with a higher virulence, deaths occurred and the percentage of animals dying increased with the virulence of the strain of *B. pestis* used. Bugs that had been starved for longer or shorter periods were used, and it was discovered that the longer the period of starvation prior to the infected meal, the longer the period that the bacilli could be recovered from the bug. The longest time after infection that *B. pestis* could be recovered by culture was eight days, but the longest period after infection that the disease could be conveyed by bite was five days. The smallest number of infected bugs used successfully to convey infection was three. The same batch of infected bugs was able to transmit the disease to two batches of animals on successive days, but failed on the third occasion.

Nuttall (1899—1900) tried on four occasions to convey the disease to mice by means of infected bugs, but failed on each occasion. He was able to recover *B. pestis* from the body of bugs five days after the infected meal.

Jordansky and Klodnitsky (1908) found that attempts to convey plague by allowing bugs to feed on a mouse sick of the disease, and before they were satisfied removing them to a healthy mouse, failed. They noted that the number of bacilli in the bug's stomach increased from the third to the sixth day after the infected meal. On the tenth day involution forms appeared, and subsequently, although the bacilli disappeared from view, they were recoverable by culture after 35 days.

Jordansky and Klodnitsky (1910) stated that, of 13 bugs fed on a pest infected mouse, two survived for 83 days and were then fed on a healthy animal. Five days later *B. pestis* was demonstrated in the bugs both by microscope and culture.

The following notes are the outcome of some rather desultory work that was performed as time and opportunity permitted during the course of a more systematic research concerning the transference of pest by fleas.

#### *Methods.*

Bugs (*Cimex lectularius*) were infected by allowing them to feed on mice that were in the comatose condition that immediately precedes death from plague. In attempting to reconvey the disease to mice by allowing the bugs to feed on them, it was found that active healthy mice eat the bugs unless some provision is made to afford the insects cover from their attacks. Mice can of course be bound, but they are restless in this condition and it was especially desired to allow the bugs a chance of undisturbed feeding while the mice slept. The usual points of attack are apparently the ears, tail or feet. A simple method of overcoming the difficulty is to bore a one inch hole in a wooden block some three or more inches long and then to make a number of saw cuts in the block so as to penetrate into the hole. A suitable tube can be arranged by cutting the bases off two solid wooden postal blocks, such as are used for despatching specimen tubes, and then nailing them together at right angles (fig. 1).

Unfortunately this plan was not devised until after such infection experiments as the time at my disposal allowed for, had been carried out. The method was found to be successful, however, in so far as by its adoption it was found possible for bugs to be kept with active mice in a cage suitable for infection experiments, and to feed freely upon them.

*The effect of infected blood upon the bugs.*

Bugs in their first instar as well as older larvae, nymphs and adults, were infected. The infected blood was the apparent cause of considerable mortality among the insects; especially was this the case with the first instar larvae<sup>1</sup>.

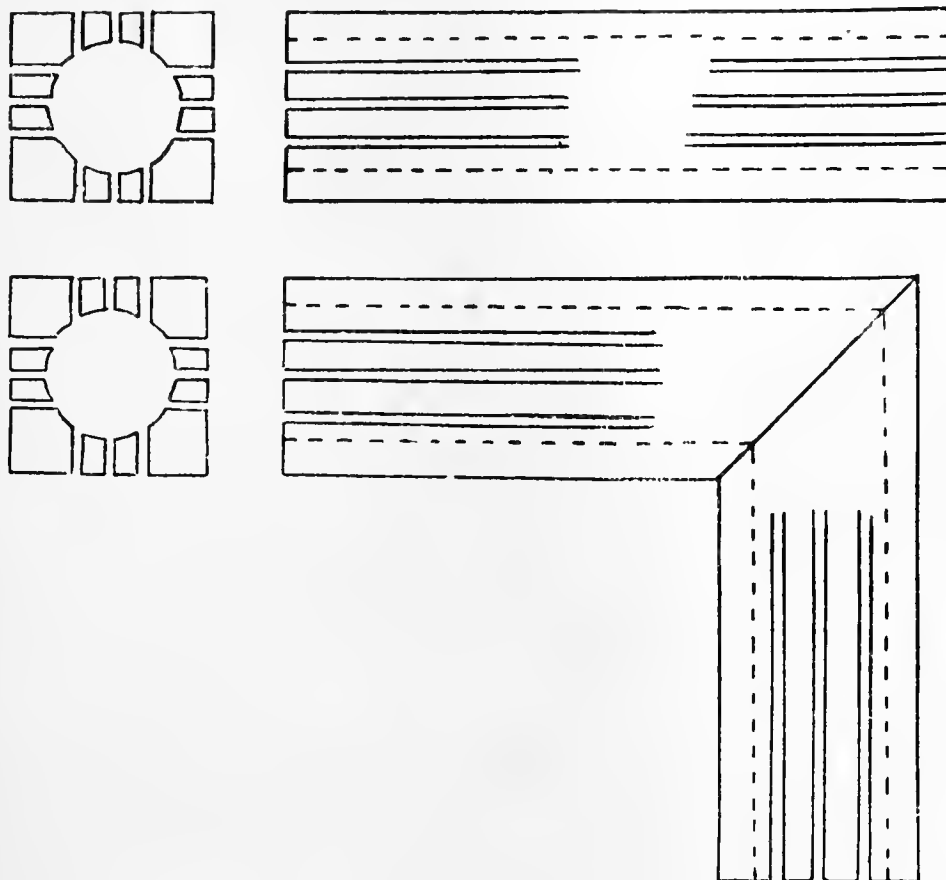


Fig. 1.

Twenty young bugs that had hatched a week or two previously were allowed to make their first meal on an infected mouse. Most of them fed to their full extent. After feeding they were placed in an entomological glass-bottomed card box and kept in a dry cupboard at 60—69° F. Two, which appeared to be injured, died the day following their meal,

<sup>1</sup> To avoid any confusion I must explain that the conditions under which bugs are kept after a full meal are important, even if the food be normal human blood. Placed at the bottom of a glass tube in a cool, moist situation, there was considerable mortality in my experience, whereas control insects kept in card boxes in a dryer and warmer situation showed no deaths. Young (first instar) bugs are more delicate in this respect than older larvae, nymphs or adults.

and an examination of the blood in their stomachs showed that it was heavily infected with *B. pestis*.

Three moulted four days after feeding, and then died; thirteen which had not moulted after seven days, were found lying at the bottom of the box, either recently dead or paralyzed. The two remaining did not moult, and were still active on the eighth day, but were found in the same paralyzed condition as the others on the tenth day.

As regards external appearances, the only unusual feature noticeable in these young bugs and the older ones which died in a similar paralyzed condition, after full meals of infected blood, was their failure to reduce their bulk, a process which is normally noticeable in healthy specimens within a few hours after a full meal. In this respect their symptoms are similar to those which accompany the death of normally fed bugs which have been kept in too cool and moist a situation after a full meal.

Smears of the stomach contents of infected bugs made at various periods after the infected meal show the following appearances. In the majority of the cases the specimens were paralyzed or dying at the time they were dissected. In only a few instances were healthy or definitely dead insects used, but the actual condition of the insects at the time of making the preparation made no apparent difference.

#### STOMACH CONTENTS TREATED AS BLOOD SMEARS.

(Stains used—*Leishman* or *Carbol-Thionin*.)

Period and temperature  
between infecting meal  
and death of bug

Remarks

##### 1 day at about 65° F.

Some large bipolar staining bacilli present, but the infection is chiefly of the "Yeast" like forms photographed by Dr Rowland (1914) in his recent paper on "The Morphology of the Plague Bacillus," figs. 16 and 17. The organisms are generally scattered in the smear—there is no clustering. Phagocytes intact and numerous. (1 specimen.)

##### 3 days at about 80° F.

A very dense growth of the small culture form (see figs. 1 and 2 in Rowland's paper). No "Yeast" forms, a few large forms and many smaller ones show bipolar staining; a tendency to cluster and clump is noticeable. There are but few phagocytes visible. (2 specimens.)

**3 days at 60° F.**

Growth much less dense than in the last—mostly consisting of the “Yeast” form of the small culture form; a few large bacilli, which show bipolar staining. No clustering. A slight tendency to form chains, but this does not progress beyond threes and fours of the slender type, phagocytes not numerous. (1 specimen.)

A second specimen shows a denser growth with the phagocytes more numerous. A third agrees with the second, but has a rather more varied assortment of form, and fewer of the “Yeast” form.

**5 days at 65° F.**

This differs but little from the 3-day specimens, but there is no trace of any “chain” tendency.

**5 days at 32° F.**

Bacteria few and scattered; all large form, often short and mostly showing bipolar staining; blood appears unaltered in structure. (1 specimen.)

**5 days at 80° F.**

Very numerous “Yeast” and short, broad bipolar staining types; a few very long and broad and a few long and narrow, both bipolar staining. A very noticeable feature are the clumps of unstainable material, presumably of autolyzed blood and unstainable bacteria. No clustering of the staining forms. (1 specimen.)

**7 days at 65° F.**

Bacteria very numerous, scattered, of the “Yeast” type, with a certain admixture of large, short bipolar staining forms, and in one specimen a small proportion of the slender bipolar forms also. Phagocytes are present, but the smears are too poor to allow of any decision as to their condition. (4 specimens.)

**8 days at 60—65° F.**

Very numerous, the greater portion of the “Yeast” form; a few pairs of the slender forms set end to end, but no actual chains. There is also a sprinkling of the large bipolar staining forms, mostly rather stumpy. Same tendency to clustering is noticeable, but no actual clumps. Phagocytes are present. (4 specimens.)

**10 days at 60—65° F.**

Large clumps composed of autolyzed blood, and masses of the bodies of bacteria which do not stain are present. Mixed with them in the clumps, and also generally distributed throughout the smear, are numerous well-stained forms, mostly of the “Yeast” type. There is also a sprinkling of large bipolar staining bacilli, for the most part rather short. The general appearance reminds one of some of the phases found in fleas, with the modification that recognizable phagocytes are present after ten days. (1 specimen.)

## 782      *Development of Bacillus Pestis in Bugs*

Period and temperature  
between infecting meal  
and death of bug

Remarks

### 10 days at 60—65° F.

A second specimen shows neither the clumps of autolyzed blood nor the non-staining bacteria, but only a general distribution of numerous "Yeast" forms and some other types. The general appearance is very different from the last. The blood has apparently been but little altered—red blood cells and phagocytes being present in numbers. (1 specimen.)

### 15 days at 60° F.

This bug had only had a small meal. The stomach contents mostly consist of unstainable material—clusters of the non-staining bacteria being mixed with the autolyzed blood. A very few and widely scattered stained forms are present and some of these show bipolar staining. No phagocytes discernible. (1 specimen.)

### 15 days at 65—70° F.

Various small faintly staining forms are present, mostly short; in many cases showing bipolar staining. A few pairs visible; recognizable phagocytes rare. (1 specimen.)

### 21 days at 28—30° F.

Large bipolar forms, many dividing and a few short chains. The bacilli few and scattered; the blood apparently unaltered structurally. (1 specimen.)

### 21 days at 65° F.

Numerous but scattered, for the most part small, inclining to the "Yeast" form, but some bipolars of small size are present. Phagocytes still easily recognizable.

### 60 days at 80° F.

The smear shows masses of small coccus-shaped bodies embedded in the autolyzed matter of which the smear is made. These bodies are not very sharp in outline and are usually unstained and of a dull greenish hue. I have, however, come across one or two small groups of similar bodies which are faintly stained. When compared with definite pest smears, one hesitates, in spite of the wide variation of *B. pestis*, to say that these minute and indistinct bodies are pest bacteria, nevertheless I suspect this to be the case and look upon them as the dead or degenerate remains of a starved culture. (1 specimen.) In this connection note the following example.

A bug that had fed on a mouse dying of pest was then fed at intervals of 3, 7, 18 and 19 days on a normal rat, which did not contract the disease. The bug died on the 40th day after the infected meals. The bacteria present in the stomach smear are very numerous and much smaller than those in the other smears, in some cases they are as minute as the bodies in the last specimen referred to above. In shape they are mostly of



either the "Culture" or "Yeast" forms, but others still, if unusually small, are of the slender type arranged in twos and threes. A few of the normal blood forms showing bipolar staining are present—these also being very small in comparison with similar forms on other smears. All of these types stain more heavily than usual. Red blood cells and phagocytes are numerous and easily discernible, but the former appear in poor condition—being much distorted in shape.

It will be seen that the characteristic form of *B. pestis* in these smears is that which Rowland (1914) has termed "Yeast" like forms and of which he shows two photographs, figs. 16 and 17. Speaking from memory, I do not recollect to have found this form in preparations of the fleas' stomachs, certainly it was never a distinctive feature as it is in the bug. As was to be expected, the number of bacteria present is to a considerable extent dependent on the period and temperature between the infected meal and the death of the bug. But, apart from this general result, to which there are some exceptions, it does not seem possible to draw any very definite conclusion as to the relationship between temperature or period and the type of *B. pestis* present or the development of the growth. There is, however, as in the flea, a definite association between the presence of unstainable bacteria and dark autolyzed material; also a suggestion that agglomerated growth may be associated with the destruction of the blood cells.

A clear, colourless fluid present in the rectum of many of the first instar bugs which were killed or paralyzed as the result of the infected meal, was squeezed out prior to the rupture of the crop. This showed no trace of *B. pestis* on microscopic examination.

#### *Sections.*

The bugs, after fixation, were embedded in clove oil celloidin and paraffin (an adaptation of Entz's method, *Arch. Protistenk*, xv. 1909, p. 98), and then stained with Haematoxylin and Eosin for tissues and blood, subsequently with Carbol-Thionin for the bacilli.

## SECTIONS OF PEST INFECTED BUGS.

*(Stained Haematoxylin, Eosin and Carbol-thionin.)*

Period and temperature  
between infecting meal  
and death of bug

Remarks

**1 day at 60—65° F. (Pl. XXXVII, fig. 1.)**

Transverse about the middle of crop (bug in first instar),  $\times 180$ . The infection is not generally diffused; it appears to be of a stratified nature, but with many small detached colonies. The dorsal area is decidedly freer than the ventral, suggesting a gravitational effect which is not, however, consistently borne out, as there is one large wave of infected blood in the dorsal area which comes in contact with the upper wall of the crop. There is a very definite association of the phagocytes in the blood with the bacteria, quite apart from any gravitational process. The growth of the bacilli must have been very rapid to have progressed to such an extent in 24 hours. Many of the sections show curiously sharp lines of demarcation between the masses of infected and uninfected blood. A belt of normal blood of remarkably even thickness, coming between a less regular one, carrying a mass of bacteria and phagocytes, and the wall of the crop may be observed. The division is as sharp as if a line had been drawn.

With a  $1/12''$  oil immersion lens (Pl. XXXVII, fig. 2) the bacilli are seen to be all of the culture form and the higher magnification also reveals numerous darkly staining bodies of varying sizes, larger than a bacterium and smaller than a red blood cell. From the almost invariable association of these particles with infected areas where phagocytes are present, it would seem definite that they are not normal to the blood or fluids of the bug's stomach. Appearances suggest that they are fragments of the nuclei of dead phagocytes.

**2 days at 60—65° F. (Plate XXXVIII.)**

Longitudinal vertical section of adult bug. The crop much distended with blood,  $\times 180$ . The central area shows very numerous patches of a dirty brown colour which contrast sharply with the eosin stained blood. These patches vary greatly in both size and shape; towards the anterior end of the crop is a very large patch of this description. The central area of this patch is fragmentary and loose in character, but towards its periphery it becomes very dense and within this dense belt large numbers of phagocytes are massed together. At its outer margin there is a more or less broken brightly staining fringe.

With the  $1/12''$  oil immersion lens (Pl. XXXVII, fig. 3) the dark patches above referred to are all found to be very similar in character, consisting of masses of small unstainable bodies which by their number and agreement in shape I take to be bacteria which do not take the stain, and fragments of varied, usually large size and irregular shaped (presumably autolyzed) blood cells or fragments of them. At the periphery of these masses there is a more or less broken fringe of stained bacilli. There

are also occasional, small patches of stained bacilli among the red blood cells quite apart from the masses of autolyzed material. The phagocytes are almost invariably confined within the autolyzed areas—the larger and presumably less recently developed being disproportionally favoured in this respect; in many instances they appear distorted and fragmentary. A further difference between the very large and the smaller patches is that the former alone show a degenerating central area. The same phenomena, mentioned in the account of the first specimen, of sharp lines of demarcation between infected and uninfected areas is apparent in this specimen as well. The appearance suggests that masses of bacteria had developed against the wall of the crop until they had attained some cohesion and then becoming shifted, uninfected blood had flowed in between them and the walls of the crop. In this specimen blood is present in the oesophagus from its entrance into the crop forward to the posterior end of the pump. A few stained bacteria are to be observed in the tube in front of its passage through the brain.

**5 days at 60—65° F.**

Longitudinal vertical section of adult bug,  $\times 180$ . The development in this specimen differs very greatly from that in the last. The red cells are very distinct, but few if any phagocytes are present in the crop, while in place of the stratified appearance after the one-day infection or the large autolyzed clumps in the last specimen, there is a much more even distribution of small patches of stained bacilli with slender ramifications. The autolyzed material consists of generally distributed specks. The slender portions of the stomach behind the crop and the gut, however, which were more or less empty in the other specimens, are in this one distended with partially digested material. Large numbers of red blood cells, more or less perfect in outline, are present in both tubes, a few only of which have taken the stain, the others are mere outlines of the same tint as the remainder of the autolyzed material.

With a  $1/12''$  immersion lens it is seen that this unstained material consists of what are probably fragments of blood cells and masses of unstained bacteria, with a few well-stained examples among them. In smaller areas the material present consists of masses of stained bacilli with a smaller admixture of unstainable ones. A few well-stained but distorted or fragmentary phagocytes are also visible in the lower stomach.

The difference between this and the last specimen in fact amounts to the segregation of the phagocytes and larger masses of autolyzed material to the lower portion of the stomach and gut on the one hand and their general distribution in the central area of the crop on the other.

It seems not impossible that such a result might follow the withdrawal of blood from the crop to the lower stomach, as no doubt the central area would respond to suction more readily than the outer layers, but the completeness of the segregation of the leucocytes arrived at in this specimen seems hardly credible by these means alone.

This specimen also shows blood in the oesophagus and the posterior end of the pumping chamber. There are a few bacilli to be found free in this

## 786      *Development of Bacillus Pestis in Bugs*

Period and temperature  
between infecting meal  
and death of bug

Remarks

blood, while in the folds and puckers formed by the walls of the oesophagus at its junction with the pump, dense clusters are to be seen from which small detached groups of bacilli are passing out through the narrow entrance into the pump.

### 8 days at 60—65° F.

Longitudinal vertical section of a first instar bug;  $\times 180$ . The development in this specimen consists chiefly of a large infected area within the crop, in addition to which there are some streaks ramifying from this centre with small detached patches. The lower, tubular portion of the stomach contains large masses of partially digested blood, which has not stained, while the contents of the crop at the posterior angle look dense and are of a yellowish colour. The leucocytes are as usual almost all crowded into the infected area.

With the  $1/12''$  immersion lens the bacteria are seen to be clustered within the central area and other infected patches, and to stain vividly. The same dark staining spots previously described in the account of the first specimen (1 day) are present within the large infected area. Their appearance in this specimen bears out the previously made suggestion that they are fragments of the nuclei of the leucocytes. The yellowish area at the extremity of the crop seems to consist of clotted blood; it is very dense and shows no structure, while the bacilli are thickly and evenly distributed, giving the patch a granular appearance.

The autolyzed material in the lower portion of the stomach is seen to consist of more or less digested blood, mixed with large clumps of unstained bacteria.

A second specimen, *cut horizontally*, shows a much weaker infection in the crop, but the posterior portion of the stomach, or a fold of the intestine is closely packed with a dense mass of vividly stained bacilli, the unstained autolyzed material being confined to a small central portion of the tube in this section. This is by far the densest and, presumably, the most rapid growth I have seen in the bug and approaches more nearly to that which occurs in the laked blood within the stomach of the flea than any of the specimens cut as sections have yet shown.

A third specimen, *cut transversely*, agrees pretty closely with the 1-day specimen as regards the development within the crop, but the tubular portion of the alimentary system is seen to be well filled with unstained material. With the  $1/12''$  objective this latter is seen to consist as usual of more or less broken down blood cells and dense masses of unstained bacteria.

A fourth specimen, *cut in longitudinal vertical direction*, agrees closely with the last in respect of the development of the infecting growth. It also shows blood to be present in the pumping chamber, which is heavily infected in places.

**34 days at 60—65° F.**

Longitudinal vertical section of a nymph. All the blood in the crop has been broken down, and there are no signs of bacilli in it. The autolyzed material in the tubular portion of the alimentary system contains numbers of small specks which differ considerably from the appearance of the unstained bacteria which give a granular appearance in the definitely infected bugs. There is, of course, a possibility that they may be connected with a previous growth of bacteria, but I have seen very similar objects among the autolyzed material in the intestines of normally fed bugs, and am of opinion that this insect was not infected at the time of death.

**74 days at 60—65° F.**

Longitudinal vertical section of adult. There is no definite evidence that this specimen was infected when it died. The autolyzed contents of the alimentary canal show numbers of the small unstained bodies which are possibly the remnants of bacteria, but beyond the fact that their size and distribution are more favourable to this view than in the last (34-day specimen) it is not possible to go with safety.

The sections do not give such clear details of the character of the individual bacilli as do smears; for the following reasons, among others: (1) Dehydration causes the bacteria to shrink whereas, in a smear, shrinkage is prevented; (2) The sections are rather thick (about 5 or 6  $\mu$ ); (3) The bacilli are thickly clustered together; (4) The staining, Haematoxylin and Thionin, is not very distinctive.

In all but the last two specimens dealt with (34 days and 74 days) the presence of unstained bacteria among the autolyzed material in the gut can be definitely determined, but with regard to these last there is considerable doubt, as the small dark bodies present approach more nearly to those seen among the detritus in the intestines of normally fed specimens of *Cimex lectularius* and *hirundinis*.

The points of most general interest appear to be the very definite association between the leucocytes and the bacteria. That this is due to the initiative of the leucocytes after the blood has been ingested, would seem to follow from the fact that they are massed within the largest and, presumably, oldest colonies, out of all proportion to the entire area infected; that is taking the smaller and scattered masses of bacilli into account as well as the large masses which are usually very few in comparison with the former.

Another interesting feature is the striking differences of development that generally obtain in the bug from that found in the flea. In the former, growth arises and tends to continue as isolated colonies, while in the flea the growth speedily loses its localized form and becomes general, often filling the entire stomach with a cohesive mass. It seems probable that this divergence is due to the speedy breaking down of the blood which takes place in the stomach of the flea, while in the bug the cellular structure of the blood would appear to be present for days as against a fraction of an hour or so in the flea. As regards the various phases of development exhibited by different bugs, this may be in large measure due to the volitional character of the bug's digestive apparatus. Verjbitzki called attention to the fact that starved bugs gave a different reaction to the more recently fed specimens; the implication being that the starved insects were the more thrifty in the use of the blood that they had ingested.

The general appearance of the specimens suggests that growth is at first rapid, but afterwards slows down and loses its vigour as it spreads outwards, leaving an exhausted area within the growing fringe, much as the small toadstools which form "fairy rings" in the turf are stated to do.

There are curious divergences in the development of the growth of colonies even in specimens of the same age which were fed on the same animal, kept under identical conditions for the same period and then fixed and sectioned under at one and the same time. Such differences must seemingly rest with the bug itself and are probably due to variations in the digestive action of the different specimens.

*Cases of infection following the bites of bugs carrying B. pestis.*

Only on two occasions did death follow from the bite of infected bugs and there was one doubtful case of infection when a rat fell sick and showed some of the symptoms of an animal suffering from pest, but it subsequently recovered.

A number of trials were made with two separate batches of infected bugs each consisting of larvae, nymphs and adults. The insects were kept in glass tubes and allowed to feed through a gauze covering on the shaved abdomen of the rat—the gauze cover being changed before each experiment. A number of rats were tried and the insects were in most cases allowed to feed on the same rat on several occasions. The result, as stated above, was one death, and one possibly infected rat which did not die, out of seven or eight rats.

The other experiment which gave a positive result was with a mouse. A batch of bugs, consisting of well-grown larvae, some nymphs and several adults were fed on a mouse just prior to its death from pest. They were then kept in a card-sided glass-bottomed box at about 60—65° F. without further opportunities of feeding. From this stock were drawn a number of the examples already discussed as smears or sections.

The survivors died off gradually until on the forty-eighth day there were only five living and one of these was very feeble. These five survivors were placed in a deep glass jar with some crumpled filter paper to afford them foothold and cover and a healthy mouse was put in. On the following morning only one of the bugs could be found, the others must have been eaten by the mouse, but the sole survivor had managed to feed.

Five days later the mouse died of typical pest. The glands of the right groin and axillae were both infected, while smears of the spleen and heart blood also contained numbers of *B. pestis*. Mice inoculated with the organisms recovered from the liver of this specimen also succumbed to the disease. Although a number of attempts were made to feed the surviving bug on mice and the ear of a guinea-pig, it could not be induced to bite and was killed when very feeble 74 days after its infected meal<sup>1</sup>.

*Discussion of the possible mechanism of infection by bugs.*

The marked difference in the development of *B. pestis* in fleas and bugs is probably due to the wide divergence in their structure and habits. The digestive processes of the flea result in a rapid destruction of both red blood cells and leucocytes, leaving the contents of its stomach very much in the condition of an artificial culture media, if we may judge from the development of the bacilli. In the bug, on the other hand, the crop would seem to serve chiefly as a reservoir for the storage of food: the blood within it remaining, so far as structural appearances go, unaltered for days after its ingestion. Sections show this organ to act mainly as a simple walled sack. At its posterior end only does the development of the epithelial cells suggest that any digestive process is possible. Whether as a consequence or as an accompanying phenomenon, the conditions within the crop of the bug do not favour the same comprehensive development of *B. pestis*, which is normal in the flea.

<sup>1</sup> See note under "Sections" of the last specimen which was killed and cut 74 days after the infecting meal.



Behind the crop in the long tubular portion of the stomach which continues back to the malpighian tubes, digestion is very evident, and in this area occasionally a more comprehensive growth takes place. As a rule, however, the digestive process seems powerful enough and sufficiently rapid to prevent any large area being occupied by an evenly staining growth of *B. pestis*.

As regards structure there is a very marked difference between fleas and bugs as to the junction of the oesophagus and stomach. Bacot and Martin (1914) show that the blocking of the intricate valvular proventriculus of the flea is a crucial factor in the mouth transmission of plague by these insects. With the bug the likelihood of any such stoppage is improbable. The large flat head of the *Cimex lectularius* accommodates a much more powerful pump than does the narrow laterally compressed head of the flea. It follows that the relative ratio of the pump to oesophagus in cross section is very different in the two insects, the dimensions of pumping chamber in the bug being very many times that of the short tubular oesophagus through which the blood passes into the crop. In conjunction with the alimentary system of the flea that of the bug might be described as valveless. Actually the oesophagus, as seen in sections, is found to be thick-walled with a folded and puckered inner lining which greatly obstructs the lumen of the tube. The appearance premises great capabilities of distension or extension under stress of circumstances, but little in the way of effective control of any inward or outward flow of blood, while I have been unable to trace any muscles or muscular attachments which appear capable of any constrictive power. The action of the crop itself, however, as it fills and distends in all directions will not only allow of the shortening of the oesophagus, but may finally, as it pushes forwards, cause the oesophagus to take up a more or less transverse or even a folded position which may be sufficiently acute to close the tube. Although the arrangement appears to be slipshod in the last degree, the pressure of the blood within the crop possibly proves effectual enough in preventing egress of the contents of the crop in practice. If it is less perfect from a mechanical point of view than the valve of the flea, there is little danger of any growth of *B. pestis* being able to effect a stoppage.

It has already been noted in the descriptions of the sections that *B. pestis* does actually multiply among the folds of the lining membrane of the oesophagus, and in one specimen small groups were to be seen in the act of passing out of the narrow entrance into the pumping chamber. The chances of any direct regurgitation of infected blood from the crop



into the wound made by the bug when feeding are lessened owing to the disparity in size between the oesophagus and the pumping chamber, as it is necessary that the latter be filled before any regurgitation can take place.

We must, however, bear in mind the fact that when a bug is disturbed during its meal that it will feed again within a short interval, and it seems not impossible that injected blood might at the moment of interruption be regurgitated through the pump and washed down into the wound at the next attempt by the flow of saliva which enters into the pharynx anterior to the pump. If such regurgitation takes place the loose character of the colonies within the crop in conjunction with the efficient mixing apparatus afforded by a powerful pump jerking fluid through a narrow tube into an elastic bag would seem to be an ideal aid to the chances of a sample of any bacteria present in the crop being mixed with the regurgitated fluid.

This explanation is, of course, mere speculation, but there seems to be no doubt that the infection which followed Verjbitzky's experiments was due to regurgitation.

In the cases I have recorded above one may possibly have arisen owing to injury to a bug by the mouse while it was feeding, but in the case of the rat it must have been due to simple regurgitation.

Rectal infection may, I think, be safely dismissed, owing to the fact that bugs, as pointed out by Martin (1913), do not defaecate during meals, as fleas are occasionally known to do, but hurry after their meal to some nook or cranny to digest at leisure.

#### *Conclusions.*

(1) That for a percentage of bugs (*Cimex lectularius*) and probably all newly hatched ones, a meal of septicaemic blood from a mouse dying of plague is fatal.

(2) Bugs which are not killed by the infecting meal are capable of carrying *B. pestis* and reinfesting mice after a period of 48 days' starvation.

(3) The development of *B. pestis* within the crop of bugs differs generally from that which takes place in the stomach of the flea in respect of its slower and looser growth, this limitation of activity being accompanied by and possibly due to the preservation of the structural character of the blood for many days after its ingestion into the crop.

(4) The absence of any definite valve between the pump and the crop, together with the looser nature of the growth within the bug, preclude the idea of such mechanical blockage as causes regurgitation and mouth infection by fleas. It may be surmised, however, that mouth infection when not caused by accidental or other injury to the bug while feeding, may be due to interruption followed by a second attempt.

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### EXPLANATION OF PLATES.

Plate XXXVII. Fig. 1. Transverse section through middle of crop of bug (*Cimex lectularius*) in first instar. Plague infection. 1 day at 60—65° F. a. Wall of crop. b. Infected blood. c. Uninfected blood. d. Phagocytes. (× 180.)

Fig. 2. Portion of Plate XXXVII, fig. 1. a. Wall of crop. b. Blood infected with plague bacilli. c. Uninfected blood. d. Phagocytes. (× 1000.)

Fig. 3. Portion of Plate XXXVIII. a. Autolyzed material containing bacilli. b. Phagocytes. c. Uninfected blood. (× 1000.)

Plate XXXVIII. Longitudinal vertical section of adult bug (*Cimex lectularius*). Plague infection. 2 days at 60—65° F. a. Integument. b. Pharyngeal muscles. c. Pharyngeal pump. d. Oesophagus filled with blood. e. Crop. f. Ant. cerebral ganglion. g. Post. cerebral ganglion. h. Ventral nerve cord. i. Phagocytes in autolyzed blood. (× 135.)

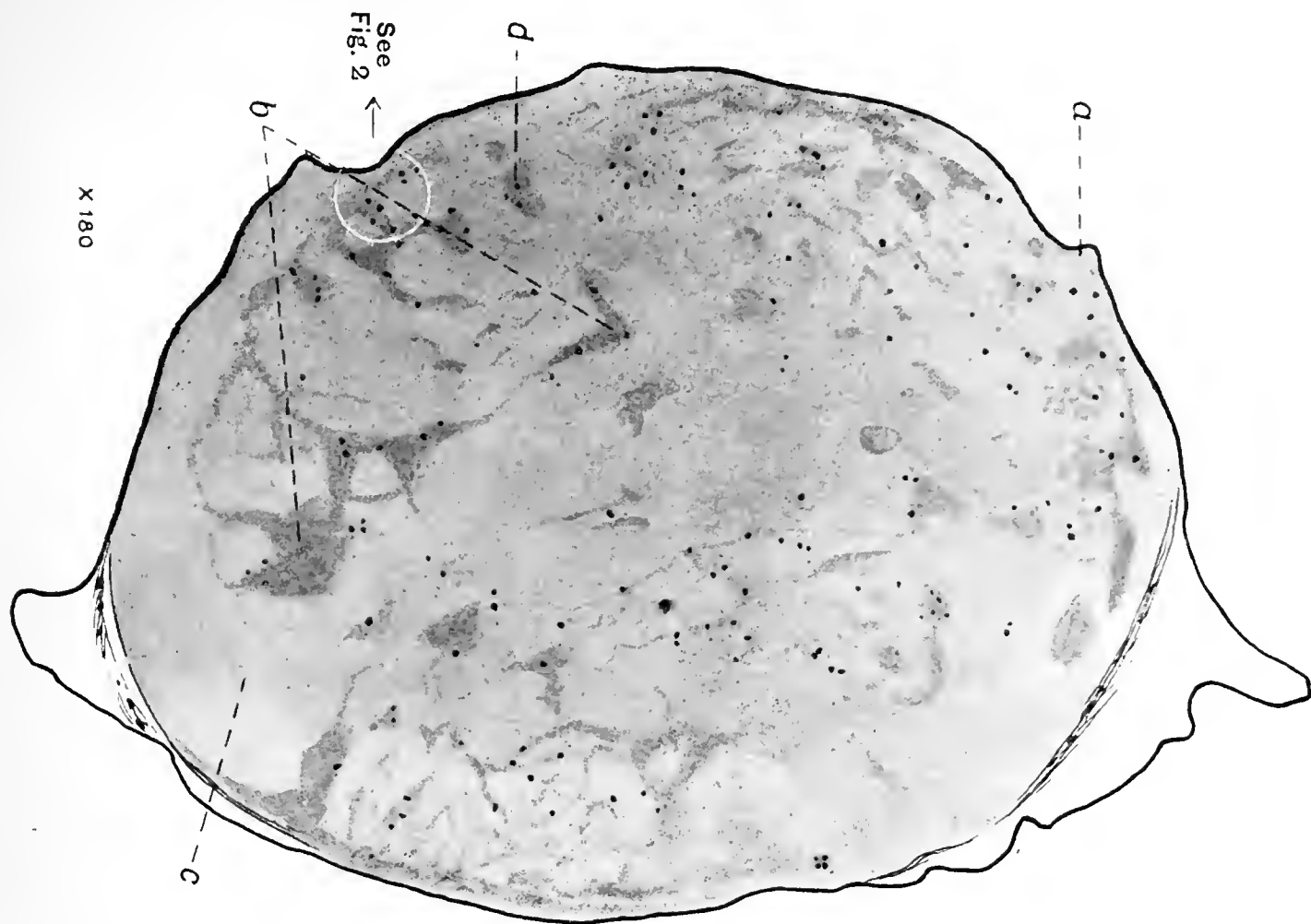


Fig. 1.

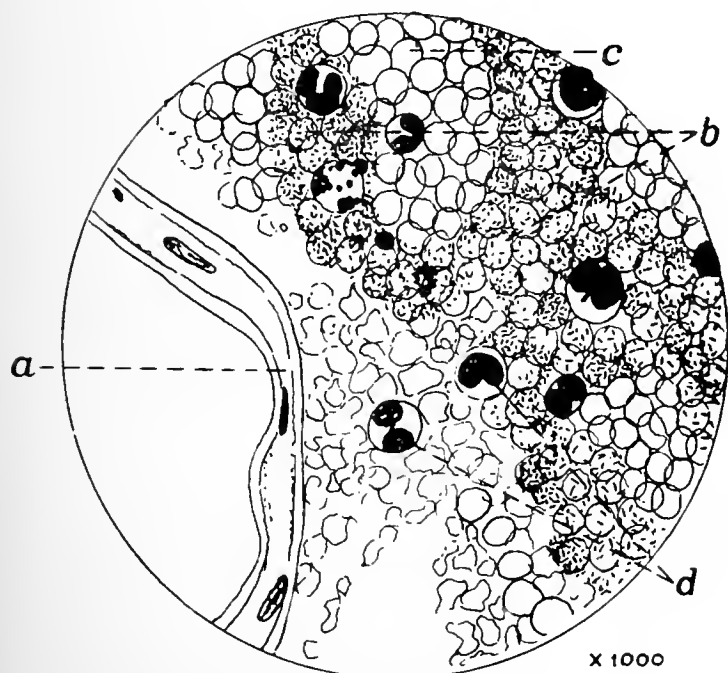


Fig. 2.

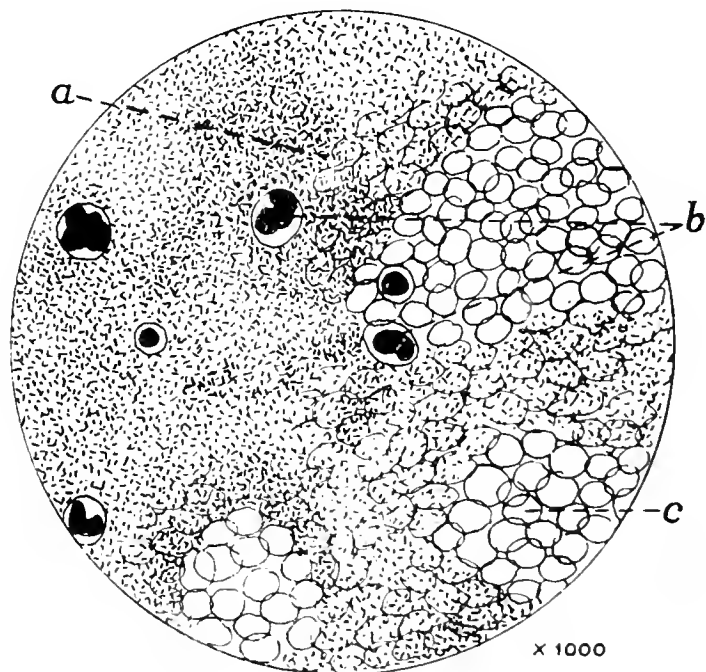
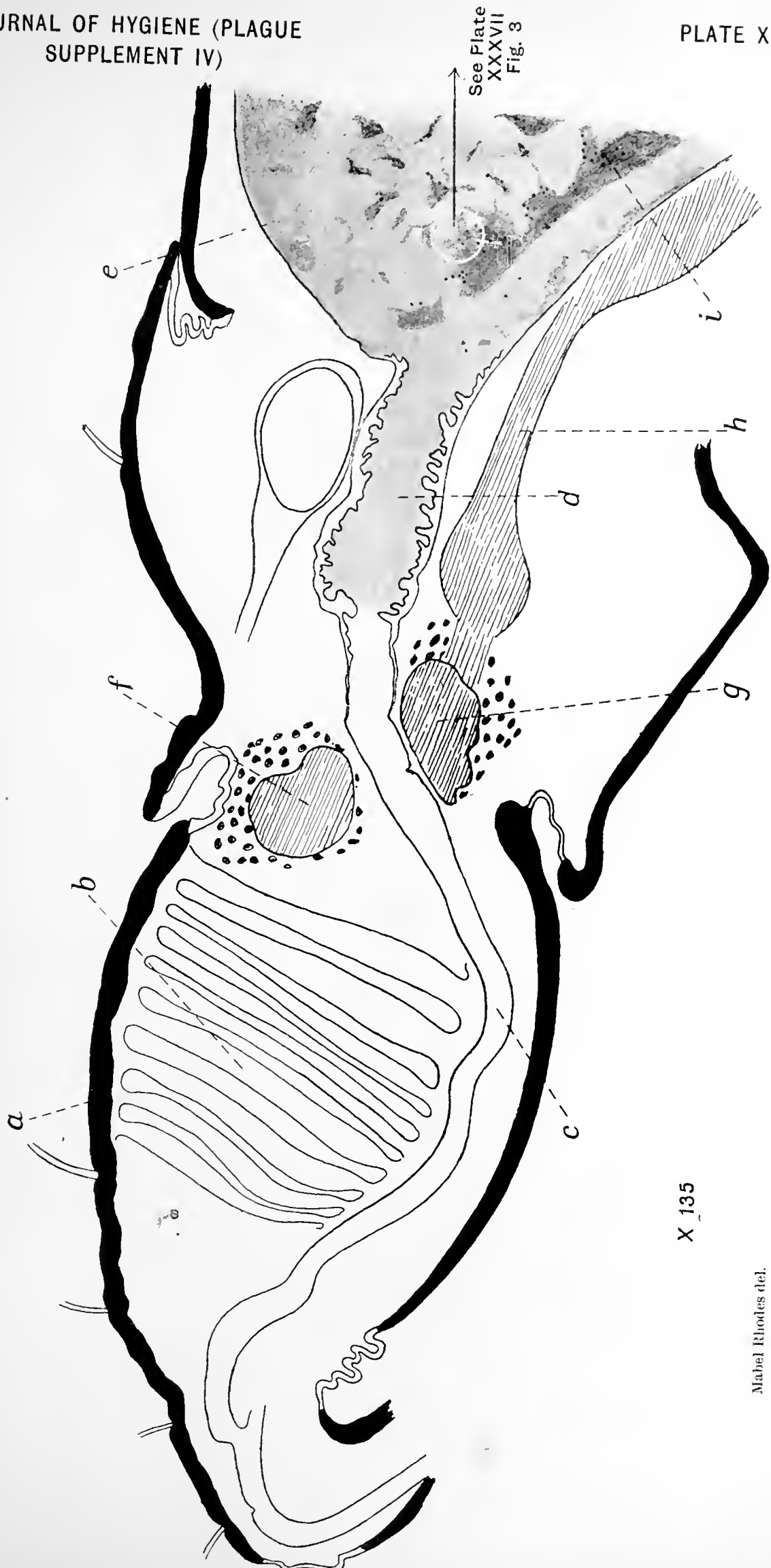


Fig. 3.





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LXXXIII. EPIDEMIOLOGICAL OBSERVATIONS IN THE  
UNITED PROVINCES OF AGRA AND OUDH, 1911—1912.

By MAJOR T. H. GLOSTER, I.M.S., AND MAJOR F. N. WHITE, I.M.S.,  
ASSISTED BY ASSISTANT-SURGEONS A. N. MUKHARJI, J. S. ROY  
CHAUDHURI AND C. C. MITRA, AND SUB-ASSISTANT-SURGEONS  
G. C. MANDAL AND MANJI RAM.

(With 4 Maps and 9 Charts.)

CHAPTER I

INTRODUCTION

At the commencement of 1911, observations were started in the United Provinces, having for their object the elucidation of several points of interest in connection with the geographical distribution of plague in these provinces. We hoped to be able to offer an explanation for the variation in the degree of intensity of plague infection that several districts of the Provinces have exhibited in the past. The significance of the problems we were called upon to investigate can be appreciated only by a study of the history of plague in this part of India. We propose therefore to preface this account of our investigations with a short description of the epidemics that have visited the United Provinces during the last decade. As a preliminary to this, some remarks will be made on the geography of the Provinces and the climatic and other conditions pertaining thereto. We will subsequently pass to an enunciation of the more remarkable of the problems that will have been disclosed, and finally describe in detail the observations made in our endeavour to solve them.

*Situation and Boundaries.*

The United Provinces of Agra and Oudh lie between  $23^{\circ} 52'$  and  $31^{\circ} 18'$  N. and  $77^{\circ} 3'$  and  $84^{\circ} 39'$  E. They are bounded on the north by Tibet, on the north-east by Nepal; on the east by the Bihar and Orissa districts of Champaran, Saran, Shahabad and Palamau; on the south by Sirguja, Rewah and other smaller Central India Native States. The Saugor district of the Central Provinces and the Jhansi district of the United Provinces have a common boundary for a short distance.

The western boundaries are formed by the States of Gwalior, Dholpur and Bhartpur and by the Punjab districts of Gurgaon, Delhi, Karnal, and Umballa. On the north-west lies the Punjab State of Sirmor. The river Jumna forms the boundary line between the Punjab and the United Provinces, and the Ganges for a short distance forms the southern boundary line separating the Bihar district of Shahabad from the eastern end of the Provinces. The Gogra and the Gandak for a short distance help to form the eastern boundary. All the rest of the boundary lines are artificial.

*Physical Geography of the Provinces.*

There are portions of four natural geographical sub-divisions of India included in these Provinces, viz.:

- (1) The Himalayan Tract which has been almost free from plague.
- (2) The sub-Himalayan Tract which has had very little plague.
- (3) The great Indo-Gangetic plain which has suffered very severely from plague.
- (4) The hill system of Central India which has had very little plague.

The Himalayan tract comprises the districts of Garhwal, Almora, Dehra Dun, a portion of Naini Tal and the Native State of Tehri. (See Map 1.)

The Indo-Gangetic plain covers more than half of the Provinces. It is for the most part fertile and closely cultivated, and in it lie the most prosperous districts in the United Provinces.

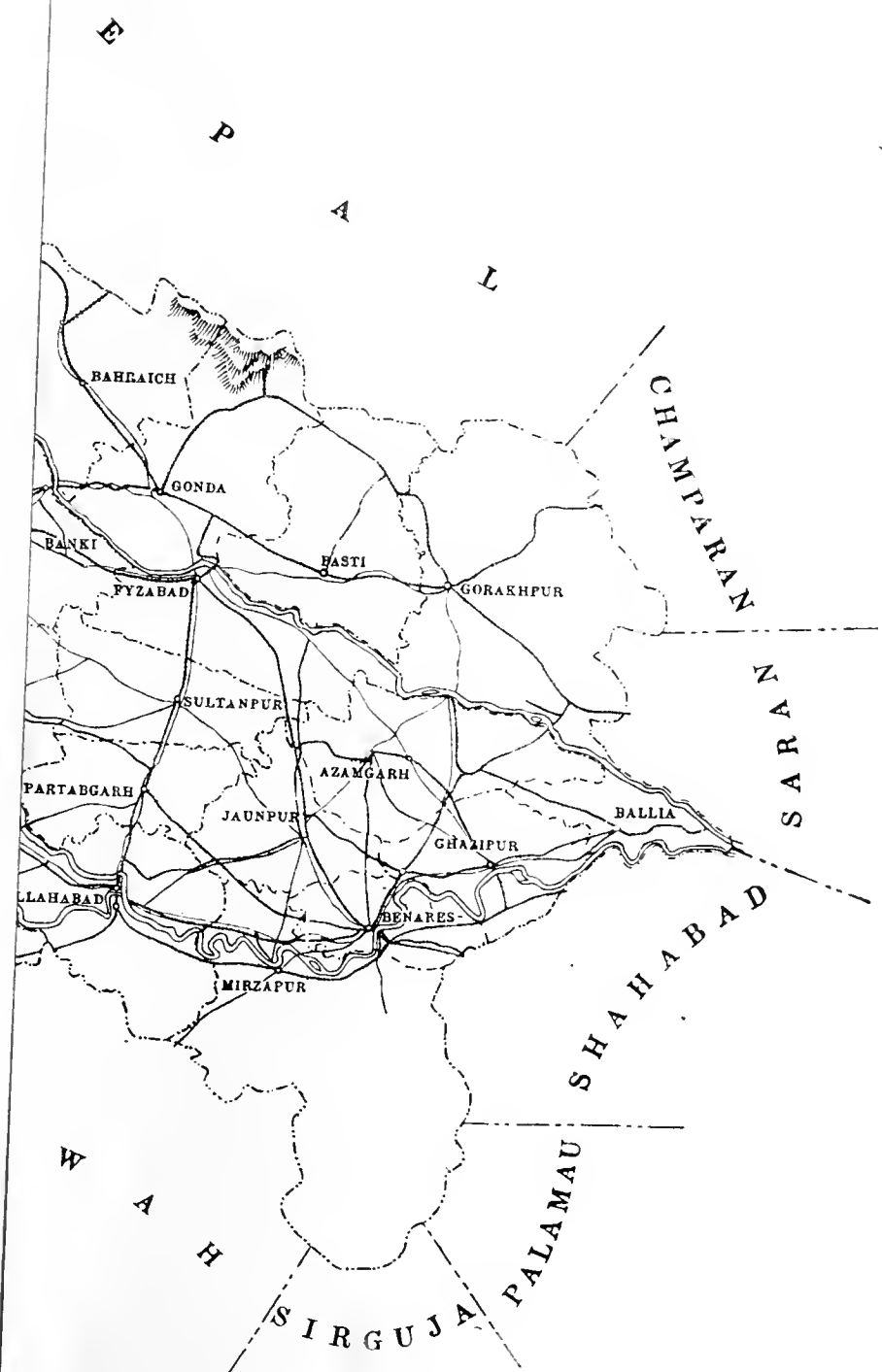
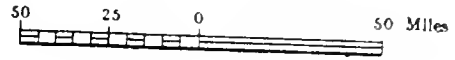
The four districts Banda, Hamirpur, Jhansi and Jalaun lying south of the Jumna form part of the Central India Plateau (see Map 1). They are comparatively rocky and infertile and are generally considered the poorest and most backward portion of the Provinces. Spurs of the Vindhya mountains break up the country in the south of these districts. The district of Mirzapur (see Map 1) which covers an area of 5200 sq. miles lies mostly on the Central India Plateau; six hundred square miles of the northern portion of this district lie in the great plain.

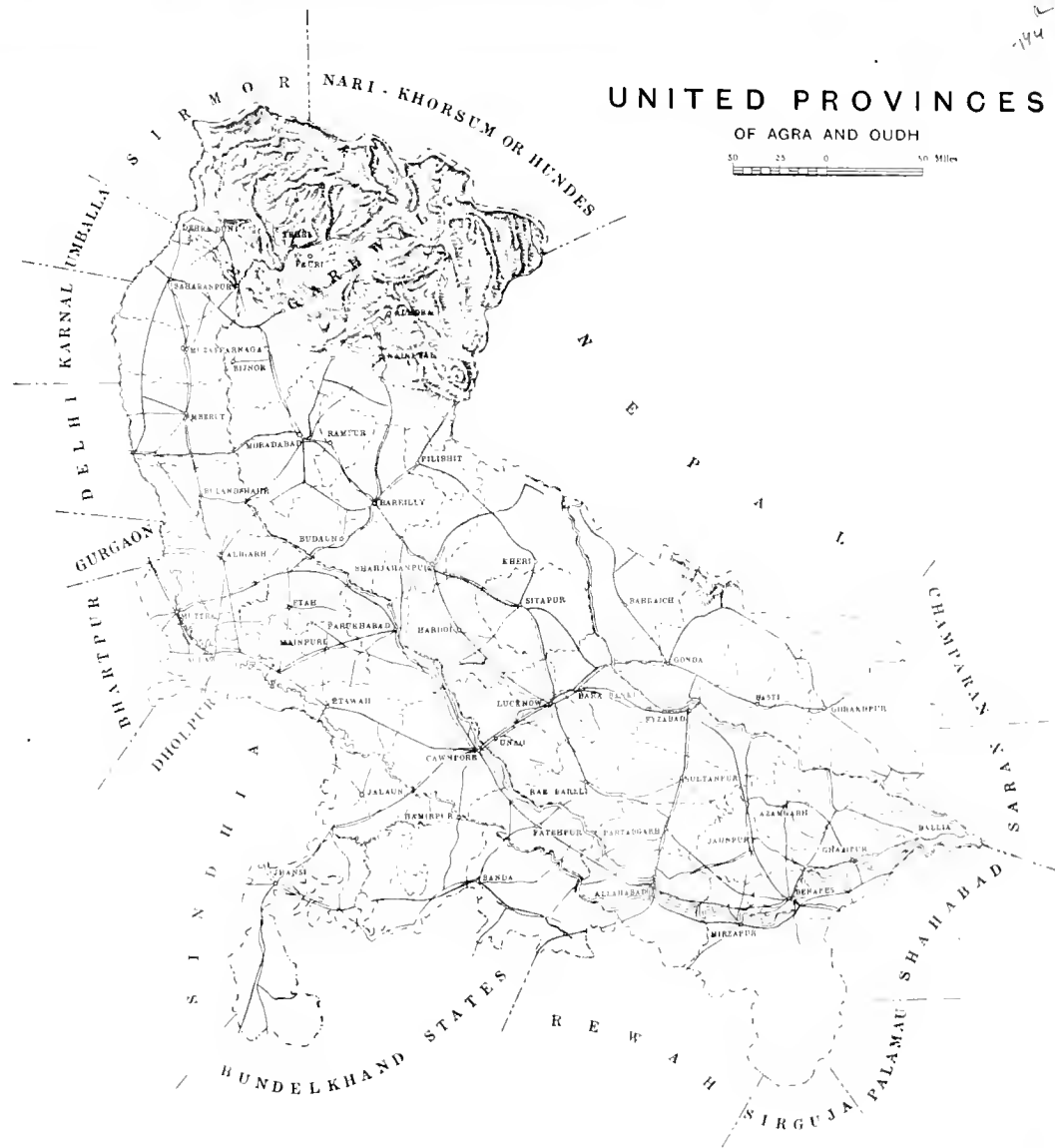
*The Climate of the Provinces.*

During the winter months of the year the mean daily temperature in the different districts of the United Provinces varies between 72° and 57° F. (see Table I). On the whole this season is dry. Such rain as does fall is distributed irregularly and is associated with storms which pass from Persia over India. These storms are generally confined to a relatively narrow belt of country which varies in position with each



# UNITED PROVINCES OF AGRA AND OUDH





storm. The rainfall associated with these storms though small in quantity is of great importance for the sowing and development of the winter crops and, as we shall show, has a marked influence on the prevalence of plague.

TABLE I. *Normal Monthly Mean of 8 a.m. Humidity.*

Stations	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Benares	84	76	58	48	53	72	86	89	84	77	80	84
Gorakhpur	82	73	57	51	63	79	86	88	84	77	76	81
Bahraich	87	78	62	51	57	75	85	88	82	76	77	82
Bareilly	83	76	58	45	48	70	85	88	82	75	77	81
Rurki	85	80	62	41	40	63	82	86	81	74	77	84
Allahabad	79	69	47	36	42	66	84	87	80	69	72	77
Lucknow	82	72	53	40	46	69	84	88	81	72	76	82
Cawnpore	79	67	46	40	46	65	82	87	80	66	66	77
Mainpuri	76	72	51	37	42	66	82	85	78	65	69	73
Agra	72	65	48	35	38	57	79	82	74	57	59	68
Meerut	77	73	56	40	41	62	79	81	76	66	70	75
Jhansi	62	53	38	28	32	56	78	82	75	52	50	57
In Bihar and Orissa												
Patna	77	68	50	51	65	77	86	87	83	73	71	75
Darbhangha	86	77	61	68	71	82	88	89	86	83	82	86

*Normal Monthly Mean of Mean between Maximum and Minimum Temperatures.*

Stations	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Benares	61.2	66.2	77.3	87.5	92.2	91.0	85.4	84.1	84.2	79.2	69.1	61.6
Gorakhpur	61.5	65.3	76.5	86.4	89.1	88.3	85.2	84.3	84.1	79.3	69.9	62.3
Bahraich	59.7	63.7	73.9	84.6	90.4	88.3	85.0	83.9	83.6	78.6	69.7	61.5
Bareilly	58.3	62.3	73.2	84.1	89.8	89.7	85.1	83.9	83.1	77.4	66.9	59.4
Rurki	57.0	60.4	70.7	82.3	88.7	89.5	85.1	83.8	82.3	75.3	65.1	57.9
Allahabad	61.1	66.0	77.5	88.1	93.2	92.1	85.5	84.2	84.2	79.0	69.1	61.7
Lucknow	60.3	64.8	75.9	86.6	91.4	90.9	85.7	84.4	84.3	78.3	68.0	60.9
Cawnpore	60.5	65.0	75.6	87.2	93.4	91.5	86.0	84.1	84.5	78.6	69.5	62.0
Mainpuri	58.9	63.6	74.2	86.5	93.6	91.3	86.1	84.6	84.5	78.7	69.2	61.2
Agra	61.0	65.4	76.9	88.1	94.3	94.4	86.8	84.9	84.9	80.4	70.1	62.4
Meerut	57.7	61.4	72.1	83.3	89.4	90.5	86.1	84.6	83.2	76.4	65.9	58.8
Jhansi	63.7	68.3	79.7	90.4	95.9	93.4	85.0	83.0	83.7	81.0	71.9	65.1
In Bihar and Orissa												
Patna	61.6	65.9	77.4	87.0	88.9	88.2	84.9	84.3	84.5	80.3	71.1	62.9
Darbhangha	62.5	65.7	75.4	84.0	86.0	85.8	84.7	84.0	83.6	79.8	71.7	64.1

During the spring months the temperature gradually rises, till by the end of May intensely hot weather prevails. These hot weather conditions are brought to an abrupt termination in June by the advance of the monsoon currents. The protracted hot weather period of drought makes it clear that the heating of the low-lying plains of India is insufficient, of itself, to produce the monsoon; it is only after the mass

of mountains lying to the north and east of India have been warmed to some critical temperature that the sudden indraught of moisture-laden winds represented by the "bursting of the monsoon" takes place. The general direction of the monsoon current is therefore towards this great mass of elevated land centering round the Himalayas. The monsoon current from the Bay of Bengal, therefore, passes partly into Burmah and partly over the north of the Bay into Bengal where it is deflected by the eastern extension of the Himalayan ranges and advances up the Gangetic plain to the east Punjab. The current from the Arabian Sea advances directly across the coast to the Himalayas.

As the humid monsoon winds pass into the interior of the country from the sea, they discharge their moisture so that the rainfall becomes less from the coast to the interior. Thus in the United Provinces, the Bay of Bengal current gives less and less rain as it passes over the eastern districts to those in the west—the average rainfall at Benares in the east of the Provinces is 40 inches per annum, at Cawnpore towards the centre it is 31 inches and at Agra in the west of the Provinces it is  $27\frac{1}{2}$  inches. Similarly the Arabian current gives less and less rain as it passes from west to east. At Jubbulpore in the Central Provinces the average rainfall is 60 inches, at Saugor further east it is 49 inches, while at Jhansi in the south-west of the United Provinces the average annual rainfall is only 37 inches. Where the two currents meet one another in the south-west of the United Provinces excessive precipitation sometimes occurs, but much depends on the strength of the two currents; if they are weak the western and southern portions of the Provinces receive little rain. The districts comprised in the Bundelkhand and to a lesser extent those in the west of the Provinces are most subject to the periodic variations of rainfall.

As the monsoon currents are forced over the Himalayas condensation rapidly takes place so that much rain falls in the sub-Himalayan and Himalayan tracts. Thus at Gorakhpur, Bahraich and Rurki on the borders of the sub-Himalayan tract, passing from east to west, the average amount of rainfall is respectively 50, 41 and 42 inches, while in the Himalayan tract the average annual rainfall at Naini Tal and at Mussoorie is respectively 102 and 97 inches.

The climatological features of the four geographical tracts then are:

- (1) In the Himalayan tract the weather is cold in the winter, the spring is comparatively cool, and heavy rain falls in the summer months.
- (2) In the sub-Himalayan tract the rainfall is heavy in the monsoon; rivers and streams are numerous and the subsoil water level is high.

Thick jungle and tall grass obtain, especially at the base of the hills, and here the land is sparsely populated by migratory forest tribes. In the portion nearer the Gangetic plain the land is well cultivated and populated.

(3) The portion of the Gangetic plain which is situated in the United Provinces enjoys a delightful climate in the winter and is densely populated and prosperous. In the late spring the weather becomes hot, while during the summer months it is warm and moist. The eastern districts of this plain are more humid and cooler than those of the west, during the hot season, but in the winter the western districts are cooler than the eastern.

(4) The districts situated on the Central India Plateau are subject to periodic excess and deficiency of rain; the humidity in the winter and spring is very low and the temperature during the hot weather is very high. The winter months are not so cold as in other parts of the Provinces.

*Area and Population.*

The area of the United Provinces is 107,164 sq. miles. There are two native states in the Provinces, Rampur and Kheri, which together cover 5079 sq. miles. The Provinces consist of forty-eight districts, grouped together into nine revenue divisions. A list of these districts with their population and their density per square mile of rural area is shown in Table II.

The population of the Provinces in 1901 was 47,691,782, but in 1911 it had fallen to 47,193,372. The table shows that the density of population per square mile of rural area is 396 for the whole Provinces, but if towns also are included the density rises to 445 persons per square mile; excluding the population of the nineteen largest towns the figure 427 per square mile is obtained. The land is thus more densely populated than any other Province in India with the exception of Bengal. The density of the population however varies greatly in the different natural sub-divisions of the Province, it is least in the Himalayan tract where a density of population of ninety-five per sq. mile is found; next to this is the hilly Mirzapur district with 186 per sq. mile and the infertile Central India Plateau with 197 persons to the sq. mile. The western sub-Himalayan districts have 409 persons per sq. mile and the eastern districts 561. The Gangetic plain is the most densely populated; here the density increases from west to east. The figures are 512 in the west, 549 in the centre and 718 in the east. In the Ballia district there are 791 people to the sq. mile.

TABLE II. *List of Districts of the United Provinces with the Distribution of Population in 1901.*

District	Population	Persons per square mile in rural area
Dehra Dun	176,195	116
Muzaffarnagar	877,188	450
Saharanpur	1,045,230	379
Meerut	1,540,175	536
Bulandshahr	1,138,101	500
Aligarh	1,200,822	502
Total Meerut division	5,977,711	436
Muttra	763,099	426
Agra	1,060,528	445
Farukhabad	925,812	465
Mainpuri	829,357	457
Etawah	806,798	435
Etah	863,948	434
Total Agra division	5,249,542	444
Bareilly	1,090,117	570
Bijnor	779,951	325
Budaun	1,025,753	461
Moradabad	1,191,993	409
Shahjahanpur	921,535	456
Pilibhit	470,339	303
Total Rohilkhand division	5,479,688	422
Cawnpore	1,258,868	440
Fatehpur	686,391	398
Banda	631,058	193
Hamirpur	458,542	179
Allahabad	1,489,358	449
Jhansi	616,759	142
Jalaun	399,726	237
Total Allahabad division	5,540,702	222
Benares	882,084	651
Mirzapur	1,082,430	186
Jaunpur	1,202,920	728
Ghazipur	913,818	607
Ballia	987,768	791
Total Benares division	5,069,020	430
Gorakhpur	2,957,074	608
Basti	1,846,153	658
Azamgarh	1,529,785	670
Total Gorakhpur division	6,333,012	636
Naini Tal	311,237	102
Almora	465,893	84
Garhwal	429,900	75
Total Kumaon division	1,207,030	84

Lucknow	793,241	512
Unao	976,639	583
Rae Bareli	1,033,761	567
Sitapur	1,175,473	498
Hardoi	1,092,834	433
Kheri	905,138	294
Total Lucknow division	5,977,086	450
Fyzabad	1,225,374	644
Gonda	1,403,195	476
Bahraich	1,051,347	379
Sultanpur	1,085,904	632
Partabgarh	912,848	613
Bara Banki	1,179,323	653
Total Fyzabad division	6,857,991	542
Total United Provinces	47,691,782	396

## PLAGUE IN THE UNITED PROVINCES

### *History of Previous Epidemics.*

The first reported case of plague in the United Provinces, within recent years, occurred on the 8th of April 1897, when a woman was found to have died of the disease in Hardwar in the Saharanpur district, a town much frequented by pilgrims. The cause of death was fully confirmed and infection was apparently acquired locally. Subsequent inquiry revealed the presence of indigenous plague in the neighbouring houses. Very energetic measures were adopted and the small epidemic of 18 cases had apparently died out by June. Infection possibly had been introduced into Hardwar, a short time before the first case was recognised, by some pilgrims who had come from Sind. In June 1897 Kankhal, a village  $1\frac{1}{2}$  miles from Hardwar, reported a mortality among the rats and, on September 16th, human plague appeared in this village. The ensuing epidemic was slight, only 64 cases being reported. It is recorded that monkeys, by which these places are very largely frequented, died in some numbers from the disease. In January 1898, plague spread to Jawalpur,  $2\frac{1}{2}$  miles distant, where 113 cases occurred and the outbreak terminated in May. Altogether during the 13 months in which infection had been present in the neighbourhood of Hardwar, there were 271 cases of plague amongst a human population of 34,000. From May 1898 to the end of the year, the United Provinces were apparently free from the disease.

The most interesting feature of this, the first epidemic of plague in these Provinces, is the limited and localised character of the outbreak

despite the enormous migratory pilgrim population which frequents Hardwar.

No death from plague was reported in 1899 till early in December of that year. In November 1899 some weavers returning from Bombay to their home in Mau Aima are said to have brought infection with them. Mau Aima is a small town of 6000 inhabitants in the Allahabad district, twenty-two miles north of Allahabad city. Adjacent villages were also attacked. The little outbreak of 156 cases (of which 121 proved fatal) came to an end in March 1900. Most energetic measures were taken to stamp out infection.

Excluding this little outbreak there were 25 imported cases reported from various districts in the United Provinces during 1900.

In 1901 plague broke out in the middle of January, simultaneously, in the Ballia and Benares districts.

In the Ballia district, Raniganj was first infected. This is a market town of some importance having intimate trade relations with the Saran district in Bengal, which was suffering very severely from plague at the time Raniganj became infected.

It is not so clear from whence Benares obtained infection. The adjoining Bengal district of Shahabad was, however, heavily infected in 1901 as well as in the preceding year. The city of Benares suffered more during this epidemic than did the rest of the Benares district. It is reported that in the city the classes trading in grain, spices, and other food-stuffs were particularly affected.

From Benares plague spread into the Jaunpur district in the month of March 1901. In this month there was also a recrudescence or, more probably, a fresh importation of the disease, in the Allahabad district. Nineteen cases then occurred in Agra which were said to have acquired their infection locally.

From this period onwards, plague spread rapidly over the greater part of the United Provinces; detailed figures relating to the number of deaths from plague each year and the number of deaths per mille of the population per annum in all the districts of the United Provinces from 1900-1911 are given in Table III.

Tables IV to XIV show the number of plague deaths each month in each year from July 1900 to June 1911 in all the districts of the Provinces<sup>1</sup>.

<sup>1</sup> Throughout this report "Plague Years," extending from July to June, will be adhered to, for the calendar year ends about the middle of the plague season in the United Provinces.



A study of Table III brings out the following points: During the eleven years in which plague was present in the United Provinces up to the end of June 1911 there had been more than one and a half million deaths from this disease among a population of forty-seven and a half millions or an average death rate per mille per annum of 3.11.

It will be observed however that in some years the disease has been much more severe than in others and in this respect the years 1904-5, 1906-7 and 1910-11 stand out prominently with death rates of 9.1, 7.1 and 7.3 respectively. Save for the first and second years of the plague, when the disease was as yet not widespread over the Provinces, the years 1907-8 and 1908-9 will be noted as light years of plague, the death rate from the disease for the whole Provinces for these years being respectively 0.56 and 0.3.

When we come to study the distribution of plague in the individual districts of the Provinces we observe that the most severely infected district is Ballia, with an average annual death rate per mille per annum for the period under review of 12.5. Closely following on this district comes Muzaffarnagar with a death rate per mille per annum of 11.2, while Muttra district comes third with a death rate of 8.78.

A closer study of plague in these districts shows that the highest death rate from plague in Ballia has been 33.01 per mille in 1909-10 and that the death rate from plague in this district has been, in every year, higher than the average death rate for the whole of the Provinces during the period under review except in 1907-8 when a death rate of only 2.66 was recorded.

The most striking features of the plague epidemic in Muzaffarnagar have been the extraordinary severity of the epidemics of 1906-7 and 1910-11 when death rates per mille per annum from plague of 56.72 and 34.05 respectively were recorded. Save for these epidemics, and to a lesser extent that of the year 1904-5 when a death rate of 18.87 was notified, the epidemics in Muzaffarnagar have not been very severe.

The epidemics in Muttra in 1904-5 and in 1907-8 are particularly noteworthy, the former because it gave the enormous death rate of 66.84 and the latter because Muttra had a death rate of 3.8 while the rest of the Provinces were comparatively free from the disease.

The comparative immunity from plague of the Bundelkhand districts, and the contrast between the severity and persistence of the disease in Cawnpore and Lucknow respectively, had also received the attention of the United Provinces Government. Sir John Hewitt,

# 802 *Epidemiological Observations in United Provinces*

TABLE III. *Plague deaths each year and their ratio per mille of population*

	1900—1		1901—2		1902—3		1903—4		1904—5		1905—6	
Districts	Plague deaths	Ratio	Deaths	Ratio	Deaths	Ratio	Deaths	Ratio	Deaths	Ratio	Deaths	Ratio
Almora	—	—	—	—	—	—	—	—	—	—	—	—
Garhwal	—	—	24	0.05	1	—	18	0.04	44	0.1	71	0.16
Naini Tal	—	—	—	—	3	—	—	—	4	—	87	0.28
Bijnor	—	—	1	—	7	—	158	0.20	7572	9.71	4840	6.20
Moradabad	—	—	—	—	1	—	1	—	6624	5.56	2386	2.00
Bareilly	—	—	—	—	6	—	312	0.30	5623	5.16	910	0.84
Pilibhit	—	—	—	—	—	—	1	—	862	1.91	280	0.6
Shahjahanpur	—	—	1	—	5	—	562	0.61	1676	1.82	761	0.82
Budaun	—	—	—	—	—	—	4	—	9625	9.38	1666	1.62
Dehra Dun	—	—	1	—	—	—	1	—	10	—	24	0.13
Saharanpur	—	—	19	0.02	563	0.54	1368	1.31	10122	9.68	2153	2.06
Muzaffarnagar	—	—	1	—	1018	1.16	5275	6.01	16554	18.87	2864	3.26
Meerut	—	—	247	0.16	3636	2.36	2937	1.91	11791	7.65	462	0.30
Bulandshahr	—	—	—	—	107	0.09	11	0.01	5392	4.73	437	0.38
Aligarh	—	—	—	—	1	—	110	0.09	18825	15.67	68	0.05
Etah	—	—	—	—	—	—	73	0.08	13077	15.14	522	0.60
Muttra	—	—	—	—	1	—	1375	1.80	51002	66.84	262	0.34
Farukhabad	—	—	—	—	1597	1.72	5504	5.95	12284	13.26	1252	1.35
Mainpuri	—	—	—	—	3	—	1764	2.13	11423	13.78	204	0.24
Agra	19	0.02	4	—	6	—	845	0.8	20876	19.68	141	0.13
Etawah	—	—	—	—	1001	1.24	3955	4.9	5198	6.44	549	0.68
Lucknow	—	—	2	—	4820	6.07	6670	8.41	10239	13.00	1997	2.52
Unao	—	—	29	0.03	5921	6.0	3364	3.44	13171	13.48	1716	1.75
Rae Bareli	—	—	2	—	241	0.23	2555	2.47	8799	8.51	1177	1.14
Sitapur	1	—	—	—	10	—	5457	4.64	312	0.26	719	0.61
Hardoi	—	—	—	—	120	0.11	1163	1.06	3025	2.77	1680	1.54
Kheri	—	—	—	—	—	—	456	0.5	317	0.35	594	0.05
Cawnpore	1	—	445	0.35	17590	14.0	7841	6.23	16448	13.06	2569	2.04
Fatehpur	—	—	270	0.4	992	1.45	1265	1.84	9239	13.46	731	1.06
Hamirpur	—	—	—	—	7	0.02	2	—	196	0.43	—	—
Banda	—	—	1	—	43	0.07	6	—	160	0.25	200	0.32
Allahabad	119	0.08	4002	2.7	14521	9.72	10937	7.32	33471	22.42	1329	0.90
Jalaun	—	—	—	—	10	0.03	971	2.43	2366	5.91	2	—
Jhansi	—	—	—	—	322	0.52	837	1.36	938	1.51	—	—
Fyzabad	—	—	1	—	2318	1.92	3687	3.06	4754	3.94	660	0.55
Gonda	—	—	1	—	134	0.09	2482	1.77	271	0.19	115	0.08
Bahraich	—	—	—	—	2	—	1243	1.18	917	0.90	816	0.77
Bara Banki	—	—	—	—	1725	1.46	11148	9.46	6325	5.36	3391	2.87
Sultanpur	—	—	—	—	7	—	628	0.58	2855	2.63	735	0.68
Partabgarh	3	—	84	0.09	1094	1.2	1652	1.81	3786	4.14	545	0.60
Gorakhpur	2	—	2580	0.87	4225	1.42	8163	2.78	7343	2.50	4837	1.64
Basti	—	—	8	—	1195	0.51	1674	0.91	4279	2.32	1864	1.01
Azamgarh	—	—	508	0.33	3145	2.0	17407	11.24	17456	11.27	6777	4.38
Ghazipur	5	—	23	0.02	556	0.6	4985	5.45	28230	30.90	884	0.97
Ballia	4181	4.2	10070	10.20	6286	6.36	14101	14.28	21037	21.29	5506	5.57
Benares	3042	3.4	129	0.14	2712	3.07	957	1.09	8695	9.86	722	0.82
Mirzapur	28	0.02	1857	1.7	129	0.1	1480	1.37	8085	7.47	473	0.44
Jaunpur	431	0.3	4916	4.08	5713	4.9	4119	3.42	12919	17.39	789	0.65
Totals	7832	0.16	25226	0.53	81794	1.71	139524	2.94	434217	9.10	60767	1.27

# *Reports on Plague Investigations in India*      803

*in the districts of the United Provinces from July 1900 to June 1911.*

1906-7		1907-8		1908-9		1909-10		1910-11		Totals	
Deaths	Ratio	Deaths	Ratio	Deaths	Ratio	Deaths	Ratio	Deaths	Ratio	Deaths	Mean ann. ratio per mille
—	—	—	—	—	—	—	—	3	—	3	—
12	0.03	—	—	—	—	—	—	20	0.04	190	0.04
6	—	42	0.13	1	—	—	—	888	2.75	1031	0.30
12933	16.58	328	0.42	3	—	2	—	2714	3.36	28558	3.33
13840	11.61	375	0.31	329	0.25	1500	1.26	9099	7.20	34155	2.60
3771	3.46	24	0.02	7	—	798	0.73	4216	3.85	15667	1.31
2465	5.24	124	0.28	—	—	252	0.54	2172	4.45	6155	1.19
2108	2.29	48	0.05	1	—	92	0.10	4037	4.27	9291	0.92
15814	15.41	334	0.33	126	0.12	2919	2.84	13103	12.44	43591	3.86
24	0.13	44	0.25	—	—	—	—	1	—	105	0.06
25238	24.15	185	0.18	23	0.02	117	0.11	3997	4.06	43785	3.81
49745	56.72	828	0.94	184	0.22	4006	4.57	27512	34.05	107987	11.20
23249	15.09	1034	0.67	59	0.04	3406	2.21	38602	25.40	85423	4.87
4077	3.58	162	0.14	—	—	910	0.80	16606	14.79	27702	2.21
5617	4.68	880	0.73	2	—	2435	2.03	6334	5.43	34272	2.51
8522	9.86	585	0.68	46	0.05	3092	3.58	5743	6.57	31660	3.33
4837	6.35	2899	3.8	981	1.30	8651	11.34	3700	5.61	73708	8.78
10461	11.29	940	1.01	465	0.50	2635	2.85	4837	5.38	39975	3.71
749	0.90	1011	1.22	502	0.61	3304	4.11	7403	9.3	26363	2.89
418	0.39	376	0.35	174	0.17	3832	3.11	10487	10.27	37178	3.19
1926	2.39	732	0.91	542	0.67	2852	3.53	4739	6.24	21494	2.42
13251	16.71	87	0.11	2	—	58	0.07	6476	8.47	43602	5.00
7977	8.16	1452	1.49	1375	1.41	11103	11.36	8934	9.80	55042	5.12
4587	4.44	771	0.75	193	0.19	2905	2.81	5162	5.08	26392	2.32
4728	4.02	2	—	—	—	—	—	880	0.77	12109	0.94
4694	4.29	376	0.34	402	0.37	1119	1.02	11977	9.80	24556	2.13
1456	1.61	19	0.02	—	—	—	—	214	0.22	3056	0.20
13520	17.38	1430	1.14	1071	0.85	6265	4.97	10036	8.79	77216	5.58
3824	5.57	605	0.88	3	—	316	0.46	3033	4.48	20278	2.69
6	—	24	0.05	—	—	—	—	56	0.12	291	0.06
3	—	—	—	1	—	1	—	2	—	417	0.06
10760	7.2	387	0.26	112	0.07	1374	0.92	4590	3.13	81602	4.97
3	—	33	0.08	—	—	51	0.13	60	0.15	3496	0.79
4	—	—	—	—	—	—	—	9	—	2110	0.31
5428	4.50	153	0.13	—	—	281	0.23	9290	8.04	26572	2.00
1180	0.84	419	0.29	6	—	293	0.21	1419	1.00	6320	0.41
360	0.3	152	0.15	—	—	239	0.23	919	0.88	4648	0.40
13440	11.41	206	0.17	77	0.06	3079	2.61	8900	8.21	48291	3.72
1763	1.63	186	0.17	90	0.08	984	0.91	10123	9.65	17371	1.46
1515	1.66	170	0.19	—	—	555	0.61	1810	2.01	11214	1.12
7555	2.57	2865	0.97	700	0.24	17363	5.87	8319	2.60	63952	1.98
1695	0.92	14	—	4	—	224	0.12	5514	3.01	16471	0.81
12712	8.21	1655	1.08	1427	0.92	24766	16.19	22921	15.35	108774	6.40
13127	14.36	1607	1.76	553	0.61	6629	7.25	15582	18.31	72181	6.08
22662	22.94	2627	2.66	4427	4.48	33403	33.01	11534	13.51	135834	12.50
1574	1.78	137	0.16	—	—	454	0.51	3470	3.87	21890	2.26
5373	4.97	107	0.10	—	—	398	0.37	7784	7.27	25714	2.16
2718	2.26	212	0.18	364	0.30	3644	3.03	19549	12.56	55374	4.18
341725	7.165	26646	0.56	14252	0.30	156307	3.28	344776	7.31	1633066	3.11

## 804 *Epidemiological Observations in United Provinces*

the then Lieutenant-Governor of the Provinces, invited the Plague Research Commission to investigate the facts outlined above which may be restated briefly in the form of the following questions.

### *The four problems which we proposed to study.*

(1) Why does Lucknow city suffer less severely and less persistently from plague than Cawnpore, which is comparatively adjacent to it?

(2) Can any explanation be offered as to why Ballia is the most persistently and severely infected district in the United Provinces?

(3) Why do the districts of the Bundelkhand, viz. Jhansi, Jalaun, Banda and Hamirpur, suffer less severely from plague than the districts of the Gangetic plain?

(4) Can any explanation be offered for the unusually severe epidemic which occurred in Muzaffarnagar in 1906-7 and in Muttra in 1904-5?

The Commission accepted Sir John Hewitt's invitation and decided, in the first instance, to establish laboratories in Lucknow and Cawnpore where facts could be collected regarding the rats and fleas of these places and where data could be accumulated which might have a bearing on the epidemics which had occurred in the Provinces as a whole. At a somewhat later date laboratories were also opened in the Ballia district and at Banda in the Bundelkhand.

Work had not long been started when we learned that the problems we had to solve for the United Provinces were only parts of much larger ones. For example the severe nature of the plague in the east of the Provinces is not confined to the Ballia district but extends into the adjacent parts of the neighbouring districts of the United Provinces as well as to the adjoining districts of Bihar and Orissa. Similarly the severe plague epidemics in Muzaffarnagar have always been synchronous with severe epidemics in the adjoining districts of the Punjab. These remarks also apply to the Muttra district. The immunity from plague of the Bundelkhand extends beyond the borders of the United Provinces. An imperfect and incomplete view of the nature of the problems we had to study will be obtained if these facts are not kept in mind.

Before stating and analysing the facts collected at the laboratories we established in Lucknow, Cawnpore, Ballia and Banda it will be well to describe the methods in vogue in the United Provinces for the reporting of plague cases and deaths.

# *Reports on Plague Investigations in India* 805

TABLE IV. *Plague Deaths month by month in the Districts of the United Provinces.*  
1900-1901.

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	Totals	Per mille
<b>Districts</b>														
<b>Kumaon</b>														
Almora	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Garhwal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Naini Tal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Rohilkhand</b>														
Bijnor	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Moradabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Barcilly	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pilibhit	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Shahjahanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Budaun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Meerut</b>														
Dehra Dun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Saharanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Muzaffarnagar	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Meerut	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bulandshahr	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Agra</b>														
Aligarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Etah	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Muttra	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Farukhabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mainpuri	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Agra	—	—	—	—	—	—	—	—	—	7	12	—	19	.01
Etawah	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Lucknow</b>														
Lucknow	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Unao	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rae Bareli	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sitapur	—	—	—	—	—	—	—	—	1	—	—	—	1	—
Hardoi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kheri	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Allahabad</b>														
Cawnpore	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fatehpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hamirpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Banda	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	—	—	—	—	—	—	—	—	18	67	34	—	119	.08
Jalaun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Jhansi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Fyzabad</b>														
Fyzabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gonda	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bahraich	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bara Banki	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sultanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Partabgarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gorakhpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Basti	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Benares</b>														
Azamgarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ghazipur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ballia	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Benares	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mirzapur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Jaunpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Totals</b>	—	—	—	—	—	—	31	454	3792	2889	659	7	7832	0.16

806 *Epidemiological Observations in United Provinces*TABLE V. *Plague Deaths.*  
1901-1902.

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Kumaon														
Almora	—	—	—	—	—	—	11	3	7	—	3	—	24	0.05
Garhwal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Naini Tal	—	—	—	—	—	—	—	—	—	1	—	—	1	—
Rohilkhand	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bijnor	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Moradabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bareilly	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pilibhit	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Shahjahanpur	—	—	—	—	—	—	—	—	1	—	—	—	1	—
Budaun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Meerut	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dehra Dun	—	—	—	—	—	—	—	—	1	—	—	—	1	—
Saharanpur	—	—	—	—	—	—	—	—	11	6	2	—	19	—
Muzaffarnagar	—	—	—	—	—	—	1	—	—	—	—	—	1	—
Meerut	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bulandshahr	—	—	—	—	—	—	—	—	66	118	62	1	247	0.16
Aligarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Agra	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Etah	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Muttra	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Farukhabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mainpuri	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Agra	—	—	—	—	—	—	—	2	1	1	—	—	4	—
Etawah	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lucknow	—	—	—	—	—	—	—	1	—	—	1	—	2	—
Unao	—	—	—	—	—	—	—	—	16	12	1	—	29	0.03
Rae Bareli	—	—	—	—	—	—	—	—	2	—	—	—	2	—
Sitapur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hardoi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kheri	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cawnpore	—	—	—	—	—	—	1	—	14	146	244	40	445	0.35
Fatehpur	—	—	—	—	—	—	—	—	79	183	8	—	270	0.4
Hamirpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Banda	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	—	4	48	84	94	312	310	858	1	—	—	—	1	—
Jalaun	—	—	—	—	—	—	—	—	1373	816	98	5	4002	2.7
Jhansi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fyzabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gonda	—	—	—	—	—	—	—	—	1	—	—	—	1	—
Bahraich	—	—	—	—	—	—	—	—	—	1	—	—	1	—
Bara Banki	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sultanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Partabgarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gorakhpur	—	—	—	—	—	—	—	—	49	20	15	—	84	0.09
Basti	—	—	—	—	—	—	60	332	832	1002	319	35	2580	0.87
Azamgarh	—	—	—	—	—	4	8	—	—	—	—	—	8	—
Ghazipur	—	—	—	—	—	—	28	105	196	100	64	11	508	0.33
Ballia	—	—	—	—	—	—	1	1	3	15	3	—	23	0.02
Benares	—	—	—	—	—	—	2303	2343	2799	1150	307	71	10070	10.2
Mirzapur	—	—	—	—	—	2	1	14	70	22	—	—	129	0.14
Jaunpur	—	—	—	—	—	—	6	322	1142	374	10	3	1857	1.7
	—	—	—	—	—	—	534	1086	2081	874	59	1	4916	4.08

*Reports on Plague Investigations in India*      807

TABLE VI. *Plague Deaths.*  
1902-1903.

	Districts	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Kumaon	Almora	—	—	—	—	—	1	—	—	—	—	—	—	1	—
	Garhwal	—	—	—	—	—	—	—	—	2	1	—	—	3	—
	Naini Tal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rohilkhand	Bijnor	—	—	—	—	—	—	—	—	—	1	2	4	7	—
	Moradabad	—	—	—	—	—	—	—	—	1	—	—	—	1	—
	Bareilly	—	—	—	—	—	—	—	1	2	3	—	—	6	—
	Pilibhit	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Shahjahanpur	—	—	—	—	—	—	—	1	1	3	—	—	5	—
Meerut	Budaun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Dehra Dun	—	2	1	1	9	20	44	22	72	279	109	4	563	0.54
	Saharanpur	—	—	—	—	28	20	97	131	237	325	169	11	1068	1.16
	Muzaffarnagar	—	—	—	—	8	83	75	162	536	1627	1058	83	3636	2.36
	Meerut	—	—	—	—	—	1	—	3	—	85	18	—	107	0.09
	Bulandshahr	—	—	—	—	—	—	—	—	—	—	—	—	1	—
	Aligarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Agra	Etah	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Muttra	—	—	—	—	20	145	286	266	470	347	59	3	1597	1.72
	Farukhabad	—	—	—	—	—	—	—	—	—	3	—	—	3	—
	Mainpuri	—	—	—	—	1	—	1	1	2	1	—	—	6	—
	Agra	—	—	—	—	—	—	65	113	327	450	43	2	1001	1.24
	Etawah	—	1	—	—	—	—	92	506	1822	2236	112	4	4820	6.07
	Lucknow	—	1	8	8	1	30	821	1315	1975	1197	197	3	5921	6
	Unao	—	—	6	21	63	323	—	5	76	143	16	1	241	0.23
	Rae Bareli	—	—	—	—	—	—	—	—	—	—	5	4	10	—
	Sitapur	—	—	—	—	—	—	1	—	—	—	16	—	—	—
	Hardoi	—	—	—	—	—	—	—	11	39	48	16	—	120	0.11
Allahabad	Kheri	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Cawnpore	14	473	1444	2758	2141	2478	2611	2287	2401	908	73	2	17590	14
	Fatehpur	—	—	4	8	19	43	25	112	368	359	54	—	992	1.45
	Hamirpur	—	—	2	1	—	—	—	—	—	4	—	—	7	0.02
	Banda	—	1	—	—	—	—	2	2	14	23	1	—	43	0.07
	Allahabad	—	110	27	77	366	1263	2661	3394	3795	2474	327	27	14521	9.72
	Jalaun	—	1	5	2	—	—	1	—	—	1	—	—	10	—
	Jhansi	—	—	—	3	92	116	23	18	30	33	7	—	322	0.52
	Fyzabad	—	—	—	—	—	—	106	327	1024	823	35	3	2318	1.92
	Gonda	—	—	—	—	—	—	—	11	33	77	12	1	134	0.09
Fyzabad	Bahraich	—	—	—	—	—	—	—	—	1	1	—	—	2	—
	Bara Banki	—	—	—	—	—	—	18	88	336	993	242	48	1725	1.46
	Sultanpur	—	—	—	—	—	—	—	—	3	4	—	—	7	—
	Partabgarh	1	9	5	5	24	152	380	221	212	73	8	4	1094	1.2
	Gorakhpur	2	2	51	77	297	668	704	920	1040	431	27	6	4225	1.42
Gorakhpur	Basti	—	—	—	—	—	106	127	204	381	328	45	4	1195	0.51
	Azamgarh	2	7	37	88	147	272	332	515	919	725	91	10	3143	2
	Ghazipur	—	—	—	—	—	—	1	55	335	129	35	1	556	0.6
	Ballia	9	32	81	192	523	809	978	955	1598	941	151	17	6286	6.36
	Benares	—	—	—	14	5	77	198	568	998	638	113	1	2712	3.07
Benares	Mirzapur	—	—	—	—	—	7	32	11	6	54	19	—	129	0.1
	Jaunpur	—	—	—	109	272	592	959	1442	1515	762	55	—	5713	4.7
	Totals	28	639	1680	3372	4017	7207	10740	13667	20571	16531	3099	243	81794	1.71

808 *Epidemiological Observations in United Provinces*TABLE VII. *Plague Deaths.*  
1903-1904.

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
<b>Kumaon</b>														
Almora	—	—	—	—	—	—	13	—	2	—	3	—	18	0.04
Garhwal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Naini Tal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Rohilkhand</b>														
Bijnor	—	—	—	—	—	—	—	1	1	43	93	20	158	0.2
Moradabad	—	—	—	—	1	—	—	—	—	—	—	—	1	—
Bareilly	—	—	—	2	—	—	—	—	82	139	81	8	312	0.3
Pilibhit	—	—	—	—	—	—	—	—	—	1	—	—	1	—
Shahjahanpur	—	—	—	—	1	—	—	1	151	358	46	5	562	0.61
Budaun	—	—	—	—	—	—	—	—	4	—	—	—	4	—
<b>Meerut</b>														
Dehra Dun	—	—	—	—	—	—	—	—	1	—	—	—	1	—
Saharanpur	1	—	—	1	3	19	54	152	339	534	203	62	1368	1.31
Muzaffarnagar	—	—	—	5	112	292	328	495	1380	1775	764	124	5275	6.01
Meerut	1	—	8	18	19	70	90	230	704	1105	621	71	2937	1.91
Bulandshahr	—	—	—	—	2	—	—	—	—	6	3	—	11	—
Aligarh	—	—	—	—	—	—	—	5	10	51	36	8	110	0.09
<b>Agra</b>														
Etah	—	—	—	—	—	—	—	—	4	45	24	—	73	0.08
Muttra	—	—	—	—	—	—	7	45	302	714	285	22	1375	1.8
Farukhabad	—	5	34	32	80	78	506	1152	2154	1264	189	10	5504	5.94
Mainpuri	—	—	—	—	—	2	55	247	678	558	208	16	1764	2.13
Agra	—	2	1	—	2	—	—	141	374	276	49	—	845	0.80
Etawah	—	—	—	3	172	318	577	540	1317	817	188	23	3955	4.9
<b>Lucknow</b>														
Lucknow	—	1	3	17	145	368	763	1156	2335	1574	300	8	6670	8.41
Unao	—	—	2	16	149	222	436	613	996	737	180	13	3364	3.44
Rae Bareli	—	—	1	—	—	101	194	454	948	667	173	17	2555	2.47
Sitapur	—	—	22	92	544	1055	1504	1110	835	260	31	4	5457	4.64
Hardoi	—	—	—	—	10	70	252	186	294	295	51	5	1163	1.06
Kheri	—	—	—	—	4	6	60	97	121	117	48	3	456	0.5
<b>Allahabad</b>														
Cawnpore	—	—	18	826	2930	1916	739	448	598	325	41	—	7841	6.23
Fatehpur	—	—	—	1	4	49	146	368	482	170	44	1	1265	1.84
Hamirpur	—	—	—	—	—	2	—	—	—	—	—	—	2	—
Banda	—	—	—	1	1	—	—	—	4	—	—	—	6	—
Allahabad	16	6	7	61	221	559	722	1829	4522	2563	412	19	10937	7.32
Jalaun	—	—	—	—	—	—	6	120	487	312	45	1	971	2.43
Jhansi	—	—	—	23	150	32	119	197	185	125	6	—	837	1.36
<b>Fyzabad</b>														
Fyzabad	—	—	—	3	89	257	676	857	1176	526	96	7	3687	3.06
Gonda	—	—	—	117	274	283	699	566	330	147	52	14	2482	1.77
Bahraich	—	—	—	—	—	—	94	228	469	397	50	5	1243	1.18
Bara Banki	—	—	—	—	—	—	2150	2404	2784	1564	236	34	11148	9.46
Sultanpur	2	—	22	107	512	1333	32	52	284	217	42	1	628	0.58
Partabgarh	—	—	—	—	—	—	266	322	538	194	21	4	1652	1.81
<b>Gorakhpur</b>														
Gorakhpur	1	8	10	64	170	761	1978	2301	2094	739	36	1	8163	2.78
Basti	1	—	—	21	45	94	268	378	553	223	81	10	1674	0.91
Azamgarh	4	8	80	229	504	1235	2966	4393	5996	1749	228	15	17407	11.24
<b>Benares</b>														
Ghazipur	—	—	—	—	1	38	170	539	2197	1726	286	28	4985	5.45
Ballia	—	3	1	91	392	991	2089	3381	4977	1907	224	45	14101	14.28
Benares	—	—	—	—	16	17	58	127	514	212	13	—	957	1.09
Mirzapur	—	—	—	7	42	60	189	195	550	385	52	—	1480	1.37
Jaunpur	—	—	2	41	108	362	504	762	1582	691	65	2	4119	3.42
<b>Totals</b>	26	33	211	1807	6795	10776	18710	26092	43354	25508	5606	606	139524	2.94



TABLE VIII. Plague Deaths.

1904-1905.

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
<b>Districts</b>														
<b>Kumaon</b>														
Almora	—	15	3	3	4	18	—	—	—	—	—	1	44	0.10
Garhwal	—	—	—	1	1	—	—	—	—	—	—	—	4	—
Naini Tal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Rohilkhand</b>														
Bijnor	—	11	25	88	241	677	1028	950	2050	1551	802	149	7572	9.71
Moradabad	—	—	—	—	—	180	738	587	1677	2092	1249	101	6624	5.56
Bareilly	—	14	35	220	694	920	1142	607	703	776	465	47	5623	5.16
Pilibhit	—	—	—	—	2	2	26	54	309	292	103	74	862	1.91
Shahjahanpur	—	3	8	13	100	209	297	246	327	294	162	17	1676	1.82
Budaun	—	—	—	—	1	52	426	679	3221	3281	1862	103	9625	9.38
<b>Meerut</b>														
Dehra Dun	—	2	2	—	—	—	1	1	3	1	—	—	10	—
Saharanpur	—	82	33	64	692	1565	2496	1648	1777	983	709	73	10122	9.68
Muzaffarnagar	2	41	176	173	788	1323	2212	1493	3414	4282	2445	205	16554	18.87
Meerut	—	6	10	71	328	702	1739	1231	2067	3035	2386	216	11791	7.65
Bulandshahr	—	—	—	—	—	—	75	145	887	2361	1773	151	5392	4.73
Aligarh	—	20	254	1412	675	1622	2225	1826	3315	4488	2623	365	18825	15.67
<b>Agra</b>														
Etah	—	—	—	60	258	616	1644	2071	3441	3590	1346	50	13077	15.14
Muttra	—	19	68	124	507	2564	7075	8286	16763	12373	3109	114	51002	66.84
Farukhabad	—	38	44	80	523	1201	2082	1530	3346	2557	841	42	12284	13.26
Mainpuri	2	11	22	50	128	356	789	1168	3666	3550	1640	41	11423	13.78
Agra	—	3	89	79	135	473	1244	2047	7038	7075	2488	205	20876	19.68
Etawah	3	6	18	40	88	309	519	619	1644	1398	516	38	5198	6.44
<b>Lucknow</b>														
Lucknow	2	53	68	95	195	918	1578	1568	3148	1970	606	38	10239	13
Unao	7	26	55	114	219	663	1572	2083	4394	3033	935	70	13171	13.4
Rae Bareli	—	32	62	154	435	885	1594	1508	2337	1281	473	38	8799	8.5
Sitapur	—	—	1	—	2	26	25	30	57	94	54	23	312	0.26
Hardoi	—	—	19	27	106	336	437	294	848	746	199	11	3025	2.77
Kheri	—	—	—	6	21	24	38	45	58	62	55	8	317	0.3
<b>Allahabad</b>														
Cawnpore	5	11	165	961	1231	1694	2039	2107	4484	2926	773	52	16448	13.06
Fatehpur	—	6	18	21	87	834	1514	1641	2908	1697	462	51	9239	13.46
Hamirpur	—	—	—	—	—	36	19	20	63	54	4	—	196	0.43
Banda	—	—	1	—	—	—	25	24	44	45	9	12	160	0.25
Allahabad	7	90	302	260	650	2580	6305	7612	7688	5987	1800	190	33471	22.42
Jalaun	—	39	178	184	186	255	281	240	521	398	81	3	2366	5.9
Jhansi	—	—	148	252	57	52	75	37	174	116	25	2	938	1.51
<b>Fyzabad</b>														
Fyzabad	3	24	40	9	44	397	864	925	1540	712	179	17	4754	3.94
Gonda	10	3	3	16	15	43	25	46	60	32	16	2	271	0.19
Bahraich	—	—	—	4	12	188	239	141	171	102	53	7	917	0.9
Bara Banki	16	51	53	42	167	546	868	843	1628	1315	651	145	6325	5.36
Sultanpur	1	5	17	31	98	265	526	490	895	428	90	9	2855	2.63
Partabgarh	—	1	—	11	133	535	886	882	915	330	87	6	3786	4.14
<b>Gorakhpur</b>														
Gorakhpur	—	78	36	31	107	483	956	946	2038	1875	705	88	7343	2.5
Basti	11	18	26	21	70	357	1000	662	1161	725	210	18	4279	2.3
Azamgarh	30	85	88	69	267	1108	2553	3181	5633	3169	1053	220	17456	11.27
<b>Benares</b>														
Ghazipur	112	495	690	616	1732	4489	5978	5534	5708	2309	532	35	28230	30.9
Ballia	141	507	397	441	789	2519	3595	3537	5456	2912	680	63	21037	21.29
Benares	—	13	31	23	114	399	1143	1752	2977	1904	332	7	8695	9.86
Mirzapur	—	79	217	253	411	1251	1600	1460	1984	664	164	2	8085	7.47
Jaunpur	9	56	65	59	209	1060	2435	2744	3761	2189	319	13	12919	17.39
<b>Totals</b>	361	1945	3468	6178	12522	34732	63929	65540	116299	91054	35067	3122	434217	9.10

810 *Epidemiological Observations in United Provinces*TABLE IX. *Plague Deaths.*  
1905-1906.

	Districts	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Kumaon	Almora	—	—	—	—	14	3	5	9	2	17	21	—	71	0.16
	Garhwal	—	—	—	—	—	1	—	15	2	—	9	—	87	0.28
	Naini Tal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rohilkhand	Bijnor	21	11	22	37	276	497	398	526	934	1508	586	24	4840	6.20
	Moradabad	3	—	—	—	1	21	101	245	768	957	279	11	2386	2.00
	Bareilly	6	3	—	—	18	73	34	43	190	372	161	6	910	0.84
	Pilibhit	6	3	4	13	28	38	59	41	47	34	7	—	280	0.60
	Shahjahanpur	—	1	4	—	26	130	120	118	180	136	45	1	761	0.82
	Budaun	2	—	9	—	32	42	21	12	130	966	443	9	1666	1.62
Meerut	Dehra Dun	—	—	—	4	7	7	2	2	1	—	—	1	24	0.13
	Saharanpur	1	—	—	8	40	91	79	153	511	875	385	9	2153	2.06
	Muzaffarnagar	—	16	3	8	66	151	70	247	483	1294	502	24	2864	3.26
	Meerut	—	—	—	—	—	—	—	—	121	271	63	1	462	0.30
	Bulandshahr	—	2	1	24	40	10	37	33	87	119	82	2	437	0.38
	Aligarh	66	2	—	—	—	—	—	—	—	—	—	—	68	0.05
Agra	Etah	21	1	2	4	6	27	—	9	72	327	53	—	522	0.60
	Muttra	67	8	1	2	—	—	51 <sup>1</sup>	18 <sup>1</sup>	—	7	108 <sup>1</sup>	—	262	0.34
	Farukhabad	1	3	66	15	39	49	77	207	345	361	88	1	1252	1.35
	Mainpuri	8	6	3	—	8	7	21	6	32	98	15	—	204	0.24
	Agra	1	—	119	6	1	12	—	—	—	—	—	2	141	0.13
	Etawah	2	12	1	17	13	38	42	99	119	156	49	1	549	0.68
Lucknow	Lucknow	1	3	3	9	13	86	152	298	662	631	126	13	1997	2.52
	Unao	—	—	4	10	59	232	185	255	574	370	27	—	1716	1.75
	Rae Bareli	—	—	7	—	26	91	177	247	330	261	37	1	1177	1.14
	Sitapur	4	19	8	11	8	49	70	57	185	260	45	3	719	0.61
	Hardoi	—	—	3	7	69	127	119	157	501	549	137	11	1680	1.54
	Kheri	—	—	—	16	29	121	124	67	99	114	24	—	594	0.65
Allahabad	Cawnpore	5	4	22	59	167	572	565	413	385	285	64	28	2569	2.04
	Fatehpur	13	3	5	7	11	58	73	83	202	216	55	5	731	1.06
	Hamirpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Banda	—	—	2	46	46	19	27	42	18	—	—	—	200	0.32
	Allahabad	66	26	17	22	40	31	68	154	328	378	192	7	1329	0.9
	Jalaun	—	—	—	—	—	—	—	1	—	1	—	—	2	—
Fyzabad	Jhansi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Fyzabad	—	—	3	9	19	36	128	99	222	132	12	—	660	0.55
	Gonda	—	—	—	1	—	4	24	39	26	3	9	9	115	0.08
	Bahraich	3	1	3	15	22	76	145	182	244	117	8	—	816	0.77
	Bara Banki	3	2	5	13	76	181	312	609	1141	901	137	11	3391	2.87
	Sultanpur	—	1	1	—	2	366	58	74	139	91	3	—	735	0.68
Gorakhpur	Partabgarh	14	3	20	23	28	42	48	85	149	122	8	3	545	0.6
	Gorakhpur	18	124	63	90	225	304	335	1142	1636	866	33	1	4837	1.64
	Basti	1	3	21	43	61	139	245	412	620	280	38	1	1864	1.01
	Azamgarh	42	143	93	60	155	493	817	1381	2184	1246	154	9	6777	4.38
	Ghazipur	2	3	—	—	3	24	67	137	277	289	78	4	884	0.97
	Ballia	4	36	26	91	109	420	628	949	1880	1215	145	3	5506	5.57
Benares	Benares	—	—	—	—	1	64	89	88	264	202	12	2	722	0.82
	Mirzapur	—	—	3	21	12	26	43	62	155	128	23	—	473	0.44
	Jaunpur	—	2	1	11	62	96	65	141	315	94	—	2	789	0.65
	Jaunpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals		386	441	546	706	1858	4854	5681	8957	16561	16309	4263	205	60767	1.27

# Reports on Plague Investigations in India 811

TABLE X. *Plague Deaths.*  
1906-1907.

	Districts	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Kumaon	Almora	3	—	—	—	—	—	—	—	—	8	1	—	12	—
	Garhwal	—	—	—	—	—	—	—	—	—	1	1	—	6	—
	Naini Tal	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rohilkhand	Bijnor	5	9	24	23	34	197	684	1364	2578	4377	3132	507	12933	16.58
	Moradabad	2	—	—	1	79	186	565	1055	2725	4991	3646	590	13840	11.61
	Barcelly	—	1	1	1	17	79	157	335	793	1324	973	90	3771	3.46
	Pilibhit	2	—	—	—	8	175	509	448	563	463	230	17	2465	5.24
	Shahjahanpur	—	3	—	5	20	91	131	162	431	729	503	33	2108	2.29
	Budaun	2	11	13	135	256	552	802	1269	3555	5971	3016	232	15814	15.41
Meerut	Dehra Dun	—	—	—	—	—	2	3	12	—	4	2	1	24	—
	Saharanpur	6	7	—	6	13	226	645	1315	5008	9056	8091	865	25238	24.15
	Muzaffarnagar	3	1	4	79	96	853	1835	3921	11481	18562	11619	1291	49745	56.72
	Meerut	—	—	—	—	82	129	365	1011	4192	9367	7163	940	23249	15.09
	Bulandshahr	1	—	—	—	1	21	33	160	741	1545	1306	269	4077	3.58
	Aligarh	—	—	73	330	215	78	74	84	720	2051	1656	336	5617	4.68
Agra	Etah	—	—	12	1	16	118	442	861	2642	2720	1503	207	8522	9.86
	Muttra	—	—	3	8	2	—	1	31	260	1639	2525	368	4837	6.34
	Farukhabad	2	—	19	27	76	293	662	1379	3447	2963	1474	119	10461	11.29
	Mainpuri	—	—	—	—	—	—	—	4	98	313	284	50	749	0.90
	Agra	—	—	1	—	—	—	—	1	28	138	220	30	418	0.39
	Etawah	1	—	4	1	2	1	35	100	443	769	499	71	1926	2.39
Lucknow	Lucknow	1	19	12	17	189	524	880	1839	4624	4049	1049	48	13251	16.71
	Unao	12	17	16	33	121	300	461	906	2295	2521	1139	168	7977	8.16
	Rae Bareli	—	25	2	7	38	110	299	536	1307	1335	826	90	4587	4.44
	Sitapur	3	16	3	51	195	436	636	618	1439	994	308	29	4728	4.02
	Hardoi	—	—	10	29	135	233	415	503	1259	1503	563	44	4694	4.29
	Kheri	4	—	8	6	31	43	166	269	411	375	129	14	1456	1.61
Allahabad	Cawnpore	20	23	45	315	630	1332	1384	1818	3387	3027	1366	173	13520	17.38
	Fatehpur	—	15	12	31	65	120	245	338	1006	1336	576	80	3824	5.57
	Hamirpur	—	—	—	—	—	—	—	1	1	2	2	—	6	—
	Banda	—	—	—	—	—	—	—	1	1	—	—	—	3	—
	Allahabad	4	2	7	11	109	241	544	1232	3070	3963	1403	174	10760	7.20
	Jalaun	—	—	—	—	2	—	—	—	—	1	—	—	3	—
Fyzabad	Jhansi	—	—	—	—	—	—	—	—	—	1	1	2	4	—
	Fyzabad	—	2	15	12	46	109	560	1218	1842	1323	286	15	5428	4.50
	Gonda	—	21	11	—	10	51	100	112	309	490	71	5	1180	0.84
	Bahraich	—	—	4	2	2	21	86	57	63	76	46	3	360	0.34
	Bara Banki	9	28	52	3	176	720	1414	2191	4404	3205	1139	99	13440	11.41
	Sultanpur	—	—	—	1	7	53	193	243	422	513	283	48	1763	1.63
Gorakhpur	Partabgarh	—	12	17	35	39	88	218	336	488	220	54	8	1515	1.66
	Gorakhpur	3	37	85	33	164	445	885	1064	1891	2108	778	62	7555	2.57
	Basti	23	111	47	46	67	88	175	308	432	310	82	6	1695	0.92
	Azamgarh	2	86	45	49	112	334	994	2023	4479	3811	713	64	12712	8.21
	Ghazipur	—	18	30	15	136	362	984	1937	4019	4140	1404	82	13127	14.36
	Ballia	24	161	201	155	714	1650	3004	4350	7353	3783	1167	100	22662	22.94
Benares	Benares	—	14	8	15	9	30	54	142	416	725	152	7	1572	1.78
	Mirzapur	9	37	84	43	35	159	362	730	1940	1788	186	—	5373	4.97
	Jaunpur	1	—	—	5	11	56	205	344	678	1029	369	20	2718	2.26
	Totals	142	676	868	1531	3961	10506	21209	36677	87243	109619	61936	7357	341725	7.16

812 *Epidemiological Observations in United Provinces*TABLE XI. *Plague Deaths.*

1907-1908.

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
<b>Kumaon</b>														
Almora	—	—	—	—	—	—	—	—	18	23	—	1	42	0.13
Garhwal	25	1	15	3	35	13	45	30	85	59	14	3	328	0.42
Naini Tal	46	4	6	1	—	—	3	1	58	185	54	17	375	0.31
<b>Rohilkhand</b>	4	—	—	—	1	6	10	—	2	1	—	—	24	0.02
Bijnor	—	—	—	—	—	34	39	14	19	17	—	—	123	0.28
Moradabad	—	—	—	—	—	1	3	10	11	13	—	—	48	0.05
Bareilly	16	—	—	3	10	6	15	43	90	114	31	6	334	0.33
Pilibhit	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Shahjahanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Budaun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Meerut</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dehra Dun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Saharanpur	21	1	1	4	35	10	38	36	33	6	—	—	185	0.18
Muzaffarnagar	11	9	7	23	43	57	34	125	210	219	83	7	828	0.94
Meerut	11	10	16	37	31	22	36	81	238	420	112	20	1034	0.67
Bulandshahr	8	—	2	—	—	6	13	8	7	84	32	2	162	0.14
Aligarh	—	—	—	—	1	21	43	141	400	230	41	3	880	0.73
<b>Agra</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Etah	14	—	24	33	15	34	31	39	202	145	45	3	585	0.68
Muttra	23	—	21	95	127	182	184	322	747	900	296	2	2899	3.8
Farukhabad	22	—	17	11	33	88	161	226	288	91	3	—	940	1.01
Mainpuri	—	—	19	20	26	59	122	176	325	221	43	—	1011	1.22
Agra	—	—	—	—	—	5	3	44	182	131	5	6	376	0.35
Etawah	—	—	43	8	52	38	20	140	267	158	6	—	732	0.91
<b>Lucknow</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lucknow	3	3	—	—	1	—	—	15	53	13	—	—	87	0.11
Unao	—	—	15	22	55	107	207	419	392	214	20	1	1452	1.49
Rae Bareli	1	—	1	—	27	75	81	212	243	109	13	9	771	0.75
Sitapur	2	—	—	—	—	—	—	—	—	—	—	—	2	—
Hardoi	2	2	1	7	9	19	52	57	138	80	9	—	376	0.34
Kheri	2	—	—	—	—	—	—	2	4	10	1	—	19	0.02
<b>Allahabad</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cawnpore	13	9	48	19	23	30	80	225	603	357	21	2	1430	1.14
Fatehpur	22	—	7	90	13	60	80	161	119	49	4	—	605	0.88
Hamirpur	—	—	—	—	—	—	6	10	8	—	—	—	24	0.05
Banda	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	6	4	18	19	2	49	52	68	109	45	15	—	387	0.26
Jalaun	—	—	—	—	—	—	—	—	19	14	—	—	33	0.08
Jhansi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<b>Fyzabad</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fyzabad	3	2	2	4	5	4	33	37	52	11	—	—	153	0.13
Gonda	1	—	—	—	—	22	60	120	189	27	—	—	419	0.29
Bahraich	4	7	14	1	—	3	18	26	56	20	3	—	152	0.15
Bara Banki	4	—	—	5	5	16	14	25	78	57	2	—	206	0.17
Sultanpur	4	—	3	—	12	20	26	40	41	26	11	3	186	0.17
Partabgarh	—	—	—	—	5	2	8	34	106	15	—	—	170	0.19
<b>Gorakhpur</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gorakhpur	17	125	153	61	83	214	332	792	743	332	11	2	2865	0.97
Basti	—	1	—	—	—	—	—	4	6	3	—	—	14	0.008
Azamgarh	16	32	45	110	20	114	203	376	566	165	6	2	1655	1.08
<b>Benares</b>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ghazipur	4	5	38	50	65	266	283	385	371	126	14	—	1607	1.76
Ballia	41	86	59	21	99	359	378	576	696	302	10	—	2627	2.66
Benares	—	8	10	15	9	10	18	45	18	4	—	—	137	0.16
Mirzapur	1	—	6	8	6	5	1	44	30	6	—	—	107	0.1
Jaunpur	2	1	28	1	4	21	9	38	68	37	2	1	212	0.18
<b>Totals</b>	358	310	622	680	863	1988	2743	5148	7895	5042	907	90	26646	0.56

TABLE XII. *Plague Deaths.*  
1908-1909.

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Districts														
Kumaon														
Almora														
Garhwal														
Naini Tal														
Rohilkhand														
Bijnor	3										1		1	—
Moradabad							3						3	—
Bareilly			2	1				4	54	143	117	8	329	0.25
Pilibhit									1		3		7	—
Shahjahanpur								1					1	—
Budaun					2	1	7	17	42	30	27		126	0.12
Meerut														
Dehra Dun									21	1	1		23	—
Saharanpur	2								63	53	66		184	0.22
Muzaffarnagar	2		1	29	1		2	3	3	7	9	2	59	—
Bulandshahr														
Aligarh								1	1				2	—
Agra								6	11	3		1	46	—
Etah	15	1	16	7	3	3	10	140	252	324	181	13	981	0.13
Muttra			3			10	30	41	107	124	114	4	465	0.50
Farukhabad					20	37	15	62	127	112	104		502	0.61
Mainpuri						28	69	24	66	40	14	3	174	0.17
Agra							27	72	188	118	137	2	542	0.67
Etawah	2						23							
Lucknow							2						2	—
Unao			5	5	12	5	69	232	408	320	293	26	1375	1.41
Rae Bareli							2	7	84	48	47	5	193	0.19
Sitapur														
Hardoi				1	2	6	67	46	144	75	56	5	402	0.37
Kheri														
Cawnpore			4	2	1	57	136	234	383	127	100	27	1071	0.85
Fatehpur						2		1					3	—
Hamirpur														
Banda														
Allahabad													1	—
Jalaun		1		3		3	23	15	32	27	8		112	0.07
Jhansi														
Fyzabad														
Gonda						1		2			3		6	—
Bahraich														
Bara Banki							4	6	28	27	12		77	0.06
Sultanpur	1				2	14	8	18	18	28		1	90	0.08
Partabgarh														
Gorakhpur	24	23	51	28	47	53	55	67	137	55	122	38	700	0.24
Basti									4				4	—
Azamgarh	4	2	4	5	14	71	199	261	496	167	181	23	1427	0.92
Ghazipur					13	7	8	152	283	63	23	4	553	0.61
Ballia	1		3		75	273	526	652	1311	754	691	141	4427	4.48
Benares														
Mirzapur														
Jaunpur		12	4		17	5	51	56	120	55	43	1	364	0.30
Totals	55	39	93	81	209	576	1336	2121	4384	2701	2353	304	14252	0.3

814 *Epidemiological Observations in United Provinces*TABLE XIII. *Plague Deaths.*

1909-1910.

	Districts	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Kumaon	Almora	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Garhwal	5	6	1	4	11	27	63	191	407	568	212	5	1500	1.26
	Naini Tal	12	3	3	1	36	72	87	74	223	230	57	—	798	0.73
Rohilkhand	Bijnor	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Moradabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Bareilly	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Meerut	Pilibhit	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Shahjahanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Budaun	1	1	2	10	21	85	200	371	953	999	262	14	2919	2.84
Agra	Dehra Dun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Saharanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Muzaffarnagar	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lucknow	Meerut	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Bulandshahr	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Aligarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Etah	—	8	14	24	66	190	307	503	954	813	204	9	3092	3.58
	Muttra	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Farukhabad	2	10	11	60	85	285	468	698	666	318	32	—	8651	11.34
Lucknow	Mainpuri	—	23	36	16	41	102	227	463	1202	918	270	6	2635	2.85
	Agra	—	8	10	14	21	78	314	633	1281	997	465	11	3304	4.11
	Etawah	1	35	31	29	76	206	299	443	987	640	90	15	3832	3.11
Allahabad	Lucknow	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Unao	8	93	62	54	174	582	2001	2792	3507	1547	236	47	11103	11.36
	Rae Bareli	2	4	6	11	87	279	634	699	861	240	76	6	2905	2.81
Allahabad	Sitapur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Hardoi	1	4	—	—	2	78	104	143	488	267	28	4	1119	1.02
	Kheri	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Cawnpore	23	34	26	38	122	454	1105	1485	2024	819	128	7	6265	4.97
	Fatehpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Hamirpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Banda	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Allahabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Jalaun	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Jhansi	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Fyzabad	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Gonda	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Bahraich	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Bara Banki	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sultanpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Partabgarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Gorakhpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Basti	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Azamgarh	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Ghazipur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Ballia	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Benares	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mirzapur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Jaunpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Allahabad	Totals	634	1751	1687	1792	1501	14230	27125	33175	43037	21585	5817	373	156307	3.28

# Reports on Plague Investigations in India 815

TABLE XIV. *Plague Deaths.*  
1910-1911.

	Districts	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals	Per mille
Meerut	Dehra Dun	—	—	—	—	—	—	—	—	—	1	—	—	1	—
	Saharanpur	—	—	1	—	4	114	328	362	894	1408	874	12	3997	4.06
	Muzaffarnagar	—	1	50	174	795	1095	2345	4326	8552	7204	2922	48	27512	34.05
	Meerut	2	3	20	219	617	643	1706	3554	9624	14150	7651	413	38602	25.40
	Bulandshahr	3	—	—	3	58	315	1033	1190	4116	6951	2835	102	16606	14.79
Agra	Aligarh	—	—	—	24	104	148	321	319	1480	3040	879	19	6334	5.43
	Muttra	4	—	—	8	148	113	253	352	690	1473	641	18	3700	5.64
	Agra	3	23	10	64	150	237	823	1210	3712	3296	925	34	10487	10.27
	Farukhabad	1	—	18	27	137	293	770	583	1575	1230	203	—	4837	5.38
	Mainpuri	—	—	1	22	117	240	526	820	2569	2481	619	8	7403	9.30
Rohilkhand	Etawah	—	19	5	6	21	110	553	689	1582	1460	285	9	4739	6.24
	Etah	4	—	4	45	130	324	649	799	1722	1718	335	13	5743	6.57
	Bareilly	—	1	3	2	51	200	597	513	1222	1323	295	9	4216	3.85
	Bijnor	—	—	2	6	110	166	194	200	546	1009	449	32	2714	3.36
	Budaun	3	5	1	31	358	736	1922	1972	4037	3259	763	16	13103	12.44
Allahabad	Moradabad	—	—	1	2	39	142	652	976	2954	3442	867	24	9099	7.20
	Shahjahanpur	—	—	—	—	2	113	538	619	1389	1088	270	18	4037	4.27
	Pilibhit	25	68	11	6	94	182	209	232	623	556	165	1	2172	4.45
	Cawnpore	4	2	31	72	238	631	2425	1853	2764	1702	306	8	10036	8.79
	Fatehpur	—	10	3	15	145	231	633	507	888	555	46	—	3033	4.48
Benares	Banda	—	—	—	—	—	—	1	1	—	—	—	—	2	—
	Hamirpur	—	—	—	—	—	—	—	14	25	17	—	—	56	—
	Allahabad	1	—	17	19	165	240	741	796	1508	912	190	1	4590	3.13
	Jhansi	—	—	—	—	—	—	—	1	1	4	3	—	9	—
	Jalaun	1	4	—	—	—	—	1	2	8	29	15	—	60	0.15
Gorakhpur	Benares	—	—	—	8	25	66	216	594	1483	997	81	—	3470	3.87
	Mirzapur	—	2	67	82	248	494	2056	2267	2140	406	22	—	7784	7.27
	Jaunpur	7	10	53	55	352	1336	4089	4500	6617	2327	203	—	19549	12.56
	Ghazipur	—	30	75	113	537	1736	3465	2733	4343	2354	195	1	15582	18.31
	Ballia	15	5	2	17	172	656	1150	1886	3991	3012	606	22	11534	13.51
Kumaon	Gorakhpur	9	84	106	100	229	454	1136	1508	2673	1851	169	—	8319	2.60
	Basti	35	81	4	19	50	246	648	749	2060	1487	135	—	5514	3.01
	Azamgarh	15	35	57	47	335	1268	4093	4516	7907	4016	556	76	22921	15.35
	Naini Tal	—	—	—	—	—	—	1	22	197	511	156	1	888	2.75
	Almora	—	—	—	—	—	—	1	—	—	2	—	—	3	—
Lucknow	Garhwal	—	—	—	—	—	—	—	4	12	4	—	—	20	—
	Lucknow	—	3	1	2	98	166	774	727	2219	1989	477	20	6476	8.47
	Unao	2	9	11	32	134	312	1071	1206	3214	2380	536	27	8934	9.80
	Rae Bareli	—	5	23	11	139	407	918	846	1646	955	212	—	5162	5.08
	Sitapur	—	—	—	—	—	—	9	27	180	473	189	2	880	0.77
Fyzabad	Hardoi	28	19	8	53	430	1198	2307	1992	3687	2074	178	3	11977	9.80
	Kheri	—	—	—	—	—	—	23	10	68	80	31	2	214	0.22
	Fyzabad	4	14	26	41	276	728	2663	2186	2274	1329	239	10	9290	8.04
	Gonda	39	43	9	19	32	98	173	198	435	349	23	1	1419	1.00
	Bahraich	—	2	3	—	7	53	60	106	287	349	52	—	919	0.88
Totals	Sultanpur	6	3	13	74	315	1151	3143	2318	2155	821	123	1	10129	9.65
	Partabgarh	—	1	—	—	15	32	236	342	743	394	47	—	1810	2.01
	Bara Banki	2	14	4	12	97	535	1282	1272	2874	2292	495	21	8900	8.21
	Totals	213	496	640	1430	6974	17209	46234	51899	103686	88760	26263	972	344776	7.31



*The Registration of Plague Cases and Deaths.*

In cities and towns that possess Municipalities the registration of cases of and deaths from infectious disease is undertaken by those bodies. In the ordinary course of events Municipalities look to the police for intimation of deaths, the police in their turn obtain their information chiefly from the municipal sweepers. Each Police Station in the larger towns keeps a record of deaths in the area of the town that the station in question serves. In times of severe plague epidemics special agencies for the earlier detection of plague cases and deaths have been organised in many towns. In the case of rural areas the duties in question devolve almost entirely on the police. In each village, the village *chowkidar* (village policeman), amongst his other duties, is responsible for the upkeep of birth and death registers. In times of plague epidemics the *chowkidar* is expected to make daily reports of plague deaths (and cases) to the *thana*, or central police station, for the area in which the village is situated. The *thanas* send daily reports of the number of cases and deaths, that have been thus reported in them, to the District Magistrate at the headquarters of their districts. The District Magistrate submits weekly reports to the Local Government. In certain districts the District Magistrate obtains his reports from the *tahsildars* (revenue officials) and not through the police. The number of cases and deaths in each infected district is published, each week, in the *Government Gazette*.

In addition monthly registers of mortality are submitted from each *thana* to the office of the Civil Surgeon of the district. These are the figures utilised by the Sanitary Commissioner in the compilation of his annual report.

It is evident that with such a system of registration as that outlined above, absolute accuracy is not to be expected. Not much reliance can be placed on the "causes of death" as chronicled in the mortality register. In times of plague epidemics there is a tendency to ascribe all deaths to plague; on the other hand it is equally likely that in the off-season plague deaths go unrecognised and so unreported. Very little reliance can be placed on the number of plague *cases*, as opposed to deaths, reported. This is obvious from the most superficial perusal of any of the Provincial plague returns. Many districts apparently take cognizance only of the deaths, for the numbers of seizures and deaths always correspond. Other districts report cases as well as deaths, but that such returns are fallacious is evidenced by the fact that



the case mortality varies from seventy (approximately correct) to one hundred per cent. In our study of past plague epidemics no account at all has been paid to the reported number of seizures, the number of deaths alone being considered. That these figures, too, contain many fallacies cannot be denied, but they can, to a limited extent, be checked by a study of the death rate from all causes, the figures for which we believe approach accuracy especially in the villages of rural areas. They are in fact sufficiently accurate for our present purposes.

## CHAPTER II

### OBSERVATIONS IN LUCKNOW

#### *Introduction.*

The reason that decided us on making Lucknow the scene of a special inquiry was an endeavour to explain the fact that plague epidemics have been neither as frequent nor severe in that city as they have in the neighbouring city of Cawnpore.

Lucknow is the capital of Oudh and one of the headquarters of the Provincial Government. It is the largest city in the United Provinces, and from the point of view of size is the fourth town in India, coming next after the three Presidency towns Calcutta, Bombay and Madras. The population of Lucknow city at the census of 1901 was 264,049 (census 1911, 240,016), representing a density of population of 12,278 persons to the square mile. Large as the population is, it is certainly considerably less than it was half a century ago.

It is particularly difficult, in a few lines, to convey to the reader a word picture of Lucknow that would give him, if unacquainted with the city, any but the vaguest of ideas on the subject. Two features of the city attract the attention of the most casual observer. In the first place it is difficult to understand how the great majority of the people find a living for, save for a paper mill, the railway workshops and a brewery, there appear to be no industrial concerns to give employment to many hands. In the second place the dilapidated condition of the houses in some quarters of the city, suggesting abject poverty, contrasts with the evidence of prosperity seen in other quarters. These conditions are perhaps more easily understood when the history of the

city is called to mind. Till as late as 1856 Lucknow was the capital of the Kingdom of Oudh and under the rule of its latter kings, whose reigns were characterised by prodigal and lavish expenditure, the city became an important centre for the manufacture of jewellery, costly fabrics and the like. The demand for these disappeared with the establishment of British rule. Many of the descendants of the nobles and dependents of the Court of Oudh still reside in Lucknow, some of them on pensions which have been subdivided in some instances till but little remains for the present beneficiaries.

Thus the overthrow of the Kingdom of Oudh was not a gain as far as the commercial prosperity of the city of Lucknow was concerned. Cotton fabrics are still manufactured on a small scale and embroidery, for which the city is famed, gives employment to a fair number of its inhabitants. Silver-ware, brass and copper industry, and pottery art-ware are the only other industries that call for mention. Small in amount as the manufactures are, for a town the size of Lucknow, they are nevertheless of more importance than is the general trade of the city. The decline of prosperity is most marked in the western portion of the town in which a very large percentage of the houses are actually in ruins. There are not many parts of the city in which overcrowding is noteworthy. Open spaces and parks abound to an unusual extent, and cultivated fields are seen almost in the centre of the municipal area. The city is now well drained (except the portion that lies to the north of the river Gumti, which is much the dirtiest part of the town), and there is also a very good pipe water supply. With its numerous fine buildings Lucknow is a city of very prepossessing appearance. Of recent years it has assumed much importance as an educational centre.

There are no very large markets or grain stores in the city. The largest grain market is in Saadatganj near the centre of the town. The next most important is in Daulatganj on the north side of the river.

#### *History of Plague in Lucknow City.*

Plague made its first appearance in the city of Lucknow in December 1902. The infection probably came from Cawnpore. The disease gradually spread and by March all six wards of the city were reporting deaths from this disease. April was the month in which the mortality was highest. Ganeshganj and Chauk, the two most densely populated wards, were those in which most plague deaths occurred. The death rate per mille from plague in the 1902-3 outbreak was 18. In the

following four years epidemics of plague occurred which gave death rates as follows, 1903-4, 15.2; 1904-5, 23; 1905-6, 1.5; and 1906-7, 26.1. From 1907 till towards the close of 1910 Lucknow remained quite free from indigenous plague.

Table XV gives the six wards into which the city is divided, the population of each, the number of plague cases that each has reported in all epidemics up to June 1910, with the plague death rate per mille for each ward.

TABLE XV.

Ward	Population	Total plague deaths	Death rate per mille
Ganeshganj	56,095	4,456	79
Chauk	56,405	4,541	80
Saadatganj	29,448	3,709	126
Wazirganj	38,938	3,186	81
Hassanganj	22,402	2,012	90
Daulatganj	32,405	1,900	58

Saadatganj has suffered more severely than any other ward of the town. It contains, as we have remarked, the largest grain stores and markets of any ward in the city. Daulatganj has suffered least; this ward is situated on the north-west side of the city, it has a low density of population, and in recent years there has been a tendency for the more wealthy inhabitants to migrate to the eastern, more populous and prosperous parts of the town. Hassanganj, the second worst infected ward of the city, gives a death ratio that is perhaps misleading. This ward includes several more or less scattered villages. If the population of these be excluded from our calculation plague has been more severe in Hassanganj than the figure 90 indicates. This ward is much behind the others in its sanitary conditions and in it are situated some of the more important grain stores of the city.

The seasonal prevalence of plague in the city has been most constant. All the epidemics have reached their height in March or April irrespective of the date on which infection appeared to have been imported into the city. The monthly figures for all plague deaths for the five epidemics is as follows: July 2; August 36; September 43; October 66; November 210; December 805; January 1668; February 2637; March 7013; April 6383; May 930; and June 11.

*Epidemic and Epizootic of Plague in Lucknow of 1910-1911.*

When the Commission started work in Lucknow in January 1911 the city was infected by plague for the first time since 1907. The first cases were reported in the month of November from Hassanganj Ward which,

as has already been mentioned, includes several more or less scattered villages on the northern side of the river Gumti, which separates Hassanganj Ward from the rest of the town.

Infection was confined to that side of the river till the month of January. In the latter end of that month infection appeared more or less simultaneously in all the five remaining wards of the city. The epidemic reached its height in the month of April, after which it rapidly declined. Table XVI gives the number of plague deaths reported from each ward of the city for each month.

TABLE XVI. *Plague Epidemic in Lucknow, 1910-1911.*

Ward	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Totals	Deaths per mille
Ganeshganj	—	—	10	15	101	29	—	155	2·7
Wazirganj	—	1	7	6	48	124	25	211	5·4
Hassanganj	10	12	23	49	160	96	1	351	15·6
Chauk	—	—	4	9	50	122	10	195	3·4
Saadatganj	—	—	1	9	84	148	32	274	9·3
Daulatganj	—	—	1	4	18	32	2	57	1·7
Totals	10	13	46	92	461	551	70	1243	5·2

It will be noted that the epidemic did not reach its height simultaneously in all the wards of the city. Those earliest infected exhibited the highest plague death rate in the month of March, those later infected not till the month of April. The disease was actually declining in the two wards Ganeshganj and Hassanganj, at the very time it was working up in the adjacent wards of the city. There was no appreciable difference between the degree of flea infestation of the rats from different wards of the city. Fleas were very numerous in March and the first half of April, after which there was a rapid decline. In the case of Hassanganj and Ganeshganj the diminished number of rats caused by the deaths from plague among them, seems to have been the most important factor in terminating the epizootic and with it the epidemic. As a matter of fact it was particularly difficult to catch rats in these wards towards the close of the epidemic.

In drawing deductions as to the severity of the epizootics from the monthly incidence of human cases it must, however, be noted that quite an appreciable percentage of the population left their dwellings during the height of the epidemic.

#### *Observations on the Rats of Lucknow.*

In January 1911 we commenced setting traps for rats in Lucknow. The rats which were caught were killed and examined and the fleas found on them counted: a description of the methods we adopted for

this purpose has been detailed at length in previous reports (*Journal of Hygiene*, Vol. VII, pp. 735 et seq.).

Between four and five hundred traps were set daily, in all parts of the city. Table XVII gives the weekly figures of the rats examined, the number of fleas found on them, and figures relating to the breeding of *Mus rattus*.

During the year the number of "rats" of all kinds brought to the laboratory amounted to 38,710. Of this number 1071 were mice (2.7 per cent.), 819 were musk rats (2.1 per cent.), the remainder, 95.2 per cent., were *Mus rattus*. No other species of rats were trapped. As in most other towns in India *Mus rattus* forms an overwhelming majority of the rat population and is, in fact, the only rat to be considered from the point of view of plague in Lucknow. From Table XVII it will be seen that the number of rats caught in Lucknow in proportion to the number of traps set was not so large as in Cawnpore (the figures for this town are shown in Table XIX), but are quite comparable with the numbers found in some other plague infected towns in which similar observations have been made (*Journal of Hygiene*, Vol. x, pp. 474-520). We would here state, as has been done in former reports, that although the method of estimating the rat population of a place, by noting the number of rats caught per one hundred traps set, is one for which we make no claim of anything like accuracy, it is nevertheless the only practical way of comparing the rat infestation of one place with another which we have been able to devise.

Much depends on the energy and enthusiasm of the rat-catching staff, as well as on the co-operation or opposition, as the case may be, of the people in whose houses traps are set. These and other factors, the relative importance of which can be but very roughly estimated in individual cases, detract much from the value of the method. In Lucknow we met with very little active opposition, but on the other hand the demand for rat traps was equally rarely evidenced.

Rats appear to be less numerous in Lucknow in the months April to August than at any other period of the year. During the year in which our observations were made, a plague epizootic of some severity was raging in the first four months, and this may account for the diminution in the rat population noted in the hot weather months. Our catches were highest in October and November.

822 *Epidemiological Observations in United Provinces*TABLE XVII. *Rats caught per every 100 traps set.*

	Week ending	Total no. of rats examined	No. of <i>Mus rattus</i> on which fleas were counted	Total no. of fleas	Average no. of fleas per <i>Mus rattus</i>	Total no. of <i>Ceratophyllus fasciatus</i>	No. of adult female rats	No. of pregnant rats	Percentage of adult females pregnant	Young <i>Mus rattus</i> (70 gms. and under)	Percentage young of total	No. of rats per 100 traps set
Jan.	28, 1911	220	214	2814	13.1	53	—	—	—	—	—	43.2
Feb.	4, „	332	316	4315	13.6	83	—	—	—	81	24.4	25.4
„	11, „	531	514	5245	10.2	160	189	67	35.4	141	26.8	32.5
„	18, „	629	602	7419	12.3	265	231	69	29.8	147	24.2	26.8
„	25, „	698	682	8345	12.2	193	320	106	33.0	156	22.6	30.8
March	4, „	641	629	8211	13.0	103	262	59	22.5	169	26.6	29.3
„	11, „	709	689	7319	10.6	73	273	102	37.3	198	28.3	31.9
„	18, „	501	485	4924	10.0	33	159	49	30.8	153	31.5	27.6
„	25, „	679	673	8136	12.0	36	265	104	39.2	103	15.3	22.8
April	1, „	634	626	6548	10.4	22	222	65	29.2	154	24.5	21.6
„	8, „	720	683	7618	11.1	16	270	99	36.6	129	18.5	23.2
„	15, „	500	469	5983	12.7	4	157	57	36.3	90	18.8	22.9
„	22, „	515	462	3998	8.7	1	154	52	33.7	99	20.7	18.8
„	29, „	536	507	4117	8.1	—	143	44	30.7	114	22.4	18.7
May	6, „	589	541	3598	6.6	—	164	53	32.3	72	13.3	18.9
„	13, „	519	475	2823	5.9	—	120	32	26.6	120	24.8	18.1
„	20, „	614	578	2281	3.9	—	161	49	30.4	117	20.3	19.1
„	27, „	598	560	2067	3.6	—	164	62	37.8	115	20.5	19.4
June	3, „	536	489	1364	2.7	—	125	37	29.6	143	29.0	20.5
„	10, „	682	627	1878	2.9	—	161	39	24.2	167	26.4	22.0
„	17, „	658	592	1398	2.3	—	161	45	27.9	148	25.0	21.0
„	24, „	720	651	1504	2.3	—	177	54	30.5	161	24.6	27.6
July	1, „	503	440	1080	2.4	—	101	25	24.7	124	28.1	18.9
„	8, „	639	583	2542	4.3	—	148	34	22.9	152	26.0	19.6
„	15, „	833	776	2557	3.2	—	229	85	37.1	230	29.0	25.8
„	22, „	703	676	2322	3.4	—	168	54	32.1	192	28.4	22.5
„	29, „	629	614	1846	3.0	—	192	65	33.8	161	26.2	20.4
Aug.	5, „	691	650	1938	2.9	—	179	44	24.5	149	22.9	21.6
„	12, „	695	666	1426	2.1	—	222	71	31.9	167	25.0	22.6
„	19, „	923	876	1676	1.9	—	258	101	39.1	324	36.9	35.9
„	26, „	917	869	2216	2.5	—	314	131	41.7	252	28.9	28.9
Sept.	2, „	974	928	2418	2.6	—	298	84	28.1	316	34.0	30.9
„	9, „	1080	1019	3253	3.1	—	318	105	33.0	312	30.6	33.9
„	16, „	924	889	3334	3.7	—	301	90	29.9	281	30.4	36.1
„	23, „	1084	1043	3687	3.5	—	337	74	21.9	366	35.0	36.1
„	30, „	788	739	2899	3.9	—	232	51	21.9	272	36.8	30.1
Oct.	7, „	947	910	5337	5.8	—	281	77	27.4	390	42.8	45.5
„	14, „	955	920	9468	10.2	—	287	87	30.3	405	44.0	40.3
„	21, „	839	810	9738	12.0	—	252	65	25.7	357	44.0	35.5
„	28, „	646	601	6467	10.7	—	183	38	20.7	258	42.9	31.5
Nov.	4, „	910	851	9724	11.4	12	263	35	13.3	344	40.2	35.6
„	11, „	806	750	8017	10.6	27	199	32	16.0	340	45.3	31.2
„	18, „	959	900	8496	9.4	89	254	32	12.5	389	43.2	37.5
„	25, „	805	754	8297	11.0	128	223	38	17.0	324	42.9	31.4
Dec.	2, „	784	730	7406	10.1	106	228	23	10.0	279	38.2	36.5
„	9, „	551	520	4900	9.4	73	155	87	10.9	212	40.7	32.5
„	16, „	635	610	4653	7.6	69	200	44	22.0	186	30.4	38.1
„	23, „	776	725	6318	8.7	101	266	40	18.4	226	31.1	30.2
„	30, „	479	454	3535	7.7	60	175	33	18.8	135	29.7	31.2
Jan.	6, 1912	413	397	3052	7.6	94	143	29	20.2	122	30.7	22.6
„	13, „	755	696	7646	10.9	258	254	53	20.8	188	27.0	29.0
„	20, „	731	701	9282	13.2	191	217	41	18.8	212	29.7	29.3
„	27, „	638	602	8395	13.9	172	229	66	28.8	163	26.8	25.2
Feb.	3, „	599	577	6659	11.5	261	228	52	22.8	174	29.9	19.2

The breeding of the Lucknow *Mus rattus*, in as far as we are able to judge from the figures denoting the percentage of adult females found pregnant, appears to be most active from the beginning of February to the end of October (see Table XVII). During these nine months about thirty per cent. of the adult females were found to be pregnant. During the remaining three months of the year the percentage fell to about eighteen. It is interesting to note that as the rat population increased (see figures for rats per 100 traps in Table XVII), recovering apparently from the effects of the plague epizootic, so the rate of breeding diminished.

*Rat Fleas in Lucknow.*

During the year 262,489 fleas were found on 35,338 *Mus rattus* examined, giving an average of 7.4 fleas per rat. Of these fleas 2683 were *Ceratophyllus fasciatus*, the remainder, with the exception of one or two specimens of *Ctenocephalus felis*, were all *Xenopsylla cheopis*, the common Indian rat flea.

Table XVII shows that there is in Lucknow a very marked seasonal prevalence of fleas ranging from 1.9 fleas per rat in the month of August, the lowest weekly figure recorded, to 13.9 in the month of January. An abrupt rise in the number of rat fleas will be noted in the month of October and an abrupt decline in the months of April and May. As has been mentioned all the plague epidemics that have occurred in Lucknow have taken place between the months of October and May, *i.e.* during the season of maximum flea infestation of the rats. The abrupt fall in the number of deaths from plague in April and May has been a constant feature of all epidemics, and can be associated with the decrease in the number of fleas on the rats, which is again influenced by the rise in the temperature and fall in humidity during these months (see Table I).

The presence of *Ceratophyllus fasciatus* on the rats of Lucknow is an interesting and noteworthy feature. So far as we are aware this flea is not found on the rats of India south of the United Provinces, with the exception of the Nilgiri Hills in Madras. South of Lucknow it appears to diminish in frequency till the Bundelkhand districts are reached where it does not occur at all. It, moreover, is essentially a flea of the colder months of the year. Not a single specimen was obtained from Lucknow rats during the months of May to October inclusive. *Ceratophyllus fasciatus* formed just over one per cent. of the total rat fleas examined in Lucknow. They are most numerous in the months of December, January and February. In the latter month they were as numerous as 3 to 4 per cent. of all rat fleas examined.



## CHAPTER III

## OBSERVATIONS IN CAWNPORE

*A Description of the City of Cawnpore.*

Cawnpore is situated on the northern bank of the Ganges forty-five miles south-west of Lucknow. As a centre for trade it stands unrivalled. The city is entirely devoted to commerce, and, in this respect, as in nearly every other, it presents a very striking contrast to Lucknow. The manufactures produced in the city are numerous and important. The city is joined up with other parts of India by rail and road in a manner that gives to Cawnpore means of communication unsurpassed in any city of the country. The Great Indian Peninsular Railway brings Cawnpore into direct communication with Bombay, the East Indian Railway with Calcutta and Delhi, and the Oudh and Rohilkhand Railway with Lucknow. By means of a metre-gauge line Cawnpore is in through communication with the Bengal and North-Western Railway system. A line is now under construction that will also bring it into direct communication with the Bundelkhand districts of Banda and Hamirpur. The Grand Trunk Road runs through the city, and in addition metalled roads run north to Lucknow, south to Hamirpur, and south-west to Kalpi in the Jalaun District. Small wonder, then, that Cawnpore should stand high as a trade centre amongst the towns of India. It has made abundant use of its opportunities.

At the census of 1901 the population of the city amounted to 191,170; it is from the point of view of population the third largest town in the Provinces (Lucknow being the first, Benares second). The population at the last census, 1911, was 157,040, in 1872 it was only 122,770.

The city is bordered on two sides by country, excavated in the past for the manufacture of bricks, and this seems seriously to hamper its extension to the south or west. The cantonments on the east, and the river on the north prevent any extension in these directions. These facts, taken in conjunction with the very rapid increase in the population, account in large measure for the degree of overcrowding, which is so marked a feature of the city of Cawnpore. A maze of winding streets, some of which are so narrow that it is



impossible to walk in them two abreast, with buildings two or three stories high, such is the main part of Cawnpore city. It presents a state of affairs that might well cause the officials, responsible for its health and well-being, to despair. The problems it presents to the sanitarian are so vast as to be all but insoluble. Short of pulling down and rebuilding, little could be done to very large portions of the town to bring them up to the requirements of modern sanitary science. The obstacles to expansion increase the difficulties enormously.

In the most crowded quarters of the town, the density of the population is 265 persons to the acre\*; here all the ground floors of the houses are taken up by shops and are not used as sleeping apartments. To walk through the worst parts of the town on a hot dusty day is an experience that will not be easily forgotten, nor is it one that creates any desire for repetition. The flies, the dirt, the squalor of it all, baffle description; we have never seen it surpassed in any other town in India. But it is not all as bad as this; there are a few good wide roads and open spaces, but these are almost entirely absent in the congested parts of the town. There is a good pipe water supply to the city; but well water appears, however, to be still very largely used for drinking purposes.

*Trade of the city.* Signs of commercial activity attract the attention of the visitor even more than the meanness of the city. Seemingly endless strings of bullock carts throng the streets leading to the principal markets, laden for the most part with grain, for Cawnpore is pre-eminent as a grain collecting and distributing centre. In the autumn months cotton is brought in in quantity, but most of the road-borne imports into Cawnpore consist of grain, oil-seeds and the like. From the figures furnished by the municipality, of the amount of money

\* The following statement founded on figures obtained in the 1911 census is of much interest as evidence of the congested state of the city. It gives the density of the population of certain of the more crowded "chuks" of Cawnpore municipality.

Chuk	Area in acres	Population	Density of population per acre
Kursawan	10	1530	153
Bultherkhana Khurd	15½	3200	206
Cooly Bazaar	15	3818	240
Misri Bazaar	7¾	1525	193
Danakhori Mohalla	8½	1960	156
Bengali Mohalla	5¼	1414	265

The total area of the municipality is 8.75 square miles; the population being 157,040. This represents a density of 28 persons per acre or 1799 per square mile.

collected by them as terminal tolls on the road-borne traffic into Cawnpore, it has been possible for us to estimate approximately that, during the twelve months ending the 29th February 1912, 265,348 bullock-cart loads of merchandise (chiefly grain) entered the city. This means an average of 725 cart-loads a day. Each cart contains from twenty to twenty-five maunds of grain (a maund is about 80 lbs. avoirdupois). In other words there are approximately six million maunds of imports per annum into Cawnpore by road alone. The rail-borne traffic is likewise enormous; during the year ending 31st March 1911, 2,169,175 maunds of grain, pulse and oil-seeds and 638,626 maunds of raw cotton were imported into Cawnpore by rail. These figures will, we think, be sufficient to cause the reader to appreciate the very great importance of the city as a trade centre.

Quite a large portion of the town can be described as one large granary. The "mohalla" or ward of the town that contains the majority of the largest stores of grain is known as Collectorganj. It is conveniently situated close to the goods yards of all the principal railway lines and consists of a large open square around which are situated buildings, most of them two-storied. The ground floor in each case is a large grain godown or store with a small room or verandah in front that is used as an office. The owners of these stores do not live in the buildings but there are dwelling rooms on the upper floors of most of them which are placed at the disposal of customers during their stay of one or two days in the city. The permanent resident population of Collectorganj is thus very small. This fact seems to us to be sufficient to account for the comparatively small death rate that has been a feature of Collectorganj in past epidemics, and which has been noted, with apparent surprise, in reports of Cawnpore epidemics.

Only a few of the godowns contain floors impervious to rats. In fact it may be said that, from the point of view of the rat, few places could be found more generous in the matter of shelter and food than the grain godowns of Cawnpore. The owners of the godowns in Collectorganj are, almost without exception, extremely averse to any measures taken with a view to diminish the rat population of their stores. There was considerable opposition to our rat trapping in this part of the city. Nearly all the grain in Cawnpore is stored in rooms above ground. Storage in underground pits, which is a common method in some parts of India, is exceptional in Cawnpore.

In addition to being a collecting and distributing centre, Cawnpore

has developed into a manufacturing town of very considerable importance. Cotton weaving and leather tanning are the two chief industries. There is also a large woollen mill. All these are very flourishing concerns. There is a government harness and saddlery factory, and an army boot and equipment factory. Sugar mills, cotton gins and presses, a brush factory and two iron foundries also give employment to large numbers of people.

It seems almost incredible that, in a city in which human beings are crowded together as they are in Cawnpore, the dwellings of the people should be shared with cattle; such however is the case. In fact the practice is extraordinarily common even for India, and adds much to the insanitary condition of the town.

*History of Plague in Cawnpore.*

In 1900 and in 1901 plague cases were imported into the city of Cawnpore, but it was not till 1902 that plague obtained a foothold in the town. From 1902 onwards no single year has passed without a plague epidemic. It was early in April of 1902 that eleven plague cases were discovered in Daulatganj Mohalla of the city. This mohalla or ward is situated in the southernmost part of the city and is of interest to us, as the reports say that each year this was the first part of the town to report plague deaths. Daulatganj is a grain storing mohalla, different varieties of pulse being chiefly stocked here. The inhabitants of this mohalla store their grain in their dwelling houses to a much larger extent than they do in Collectorganj and the other more important grain mohallas of the city. Between April, when the first cases were discovered in this mohalla, and the end of July, 1902, 452 deaths were reported from the city. The greatest number of deaths reported in any one day was twenty-one. Deaths were reported from all parts of the city. By the end of July the disease had almost died out.

Table XVIII shows the number of deaths from plague in Cawnpore in each month since the close of this, the first small epidemic. The total number of reported deaths in each epidemic, as well as the death rate per mille per annum calculated on the population (1901 census), are indicated.

This table presents many features of interest. It will be noted that the first epidemic reached its height as early as October, the next two in November, while those of the two following years were yet a month later, in December. The three epidemics of 1907-8, 1908-9, and 1909-10

828 *Epidemiological Observations in United Provinces*

did not work up to their height till March, thus approximating to the type of plague epidemic in the United Provinces taken as a whole. The epidemic of 1910-11 reverted to the earlier type, reaching its height in January.

TABLE XVIII. *Plague in Cawnpore.*

Month	1902-3	1903-4	1904-5	1905-6	1906-7	1907-8	1908-9	1909-10	1910-11	Totals
July	—	—	2	—	—	—	—	—	—	2
August	475	—	9	3	2	—	—	1	—	490
September	1731	35	151	21	20	—	2	1	—	1961
October	3009	610	835	56	159	—	4	4	13	4690
November	1554	2614	954	175	595	—	8	24	47	5971
December	473	1537	907	488	801	10	20	81	192	4509
January	70	279	523	457	477	19	105	131	881	2942
February	78	122	139	423	239	69	175	156	581	1982
March	102	129	112	224	180	298	358	206	401	2010
April	63	35	210	120	155	153	99	27	70	932
May	33	6	138	36	60	5	10	1	—	289
June	13	—	20	—	9	—	—	—	—	42
Totals	7601	5367	4000	2003	2697	554	781	632	2185	25820
	44	31	23	11.6	15.6	3.2	4.5	3.7	11.4	per mille

It is also noteworthy that the later type of epidemic has been uniformly milder than the earlier type. The epidemics have in fact exhibited a tendency to become both milder and later, with the exception of that of 1910-11 which was both earlier and more severe than its immediate predecessors.

*Observations on the Rats and Fleas of Cawnpore.*

Observations were made in Cawnpore on exactly similar lines to those in Lucknow and were carried out simultaneously with the latter. Between four and five hundred traps were put down daily and an average of a thousand rats a week examined throughout a complete year. The results of our trapping and rat and flea examination are given in Table XIX.

During the year under review 52,365 rats were caught. Of this number the very large majority (51,501) were *Mus rattus*. An examination of Table XIX shows that the number of rats caught for every 100 traps set, gradually increased from 16 in the beginning of March 1911, that is when the epidemic of plague of that year was rapidly declining, to as many as sixty during the latter half of September and beginning of October. Thereafter, with the advent of a fresh epidemic, the number of rats caught per 100 traps set declined to about 34 in February 1912. The rapid increase in the rat population at the close

TABLE XIX.

	Week ending	Total no. of rats examined	No. of <i>Mus rattus</i> on which fleas were counted	Total no. of fleas	Average no. of fleas per <i>Mus rattus</i>	Total no. of <i>Ceratophyllus fuscatus</i>	No. of adult female <i>Mus rattus</i>	No. of pregnant <i>Mus rattus</i>	Percentage of adult females pregnant	Young <i>Mus rattus</i> (70 gms. and under)	Percentage young of total	No. of rats per 100 traps set
Feb.	25, 1911	489	443	7129	16.1	12	181	73	40.3	87	19.5	29.7
March	4, "	283	274	3564	13.0	26	123	45	36.6	81	22.5	16.0
"	11, "	580	553	7072	12.7	9	225	82	36.4	204	27.7	22.4
"	18, "	489	437	5473	12.5	0	190	78	41.0	116	23.6	21.9
"	25, "	1286	1270	17967	14.1	7	518	237	45.7	376	29.5	32.7
April	1, "	1319	1318	19820	15.0	3	534	249	46.6	345	26.0	40.2
"	8, "	991	985	14441	14.6	1	420	143	34.0	232	23.4	33.9
"	15, "	889	886	13357	15.0	0	346	123	35.5	237	26.5	33.2
"	22, "	522	521	5801	11.1	0	219	78	35.6	137	21.4	28.5
"	29, "	765	756	8779	11.6	0	288	129	44.8	250	28.6	30.8
May	6, "	814	804	6915	8.6	0	270	121	44.8	316	37.7	29.0
"	13, "	1051	1044	6432	6.1	0	347	123	35.4	427	40.5	37.1
"	20, "	910	897	4377	4.9	0	290	99	34.1	377	40.9	32.4
"	27, "	966	934	4415	4.7	0	332	117	35.2	341	35.3	32.9
June	3, "	896	876	3916	4.5	0	277	93	33.6	346	38.7	30.3
"	10, "	1317	1257	5007	3.9	0	465	137	29.4	425	32.2	44.8
"	17, "	1260	1254	3960	3.2	0	433	153	35.3	447	35.2	44.4
"	24, "	840	834	2441	2.9	0	242	89	36.7	368	43.6	34.5
July	1, "	956	862	2848	3.3	0	283	103	36.4	410	33.4	41.8
"	8, "	1171	1164	4225	3.6	0	378	120	31.7	417	35.4	41.2
"	15, "	1368	1360	4026	2.9	0	465	141	30.3	467	34.1	48.5
"	22, "	1314	1310	3391	2.5	0	430	145	33.7	469	35.6	47.9
"	29, "	1373	1209	4618	3.8	0	477	172	36.1	457	33.3	44.3
Aug.	5, "	973	971	4497	4.6	0	328	116	35.3	342	35.1	40.3
"	12, "	1310	1194	4990	4.1	0	462	151	32.6	422	32.2	45.5
"	19, "	1419	1409	5763	4.1	0	508	164	32.2	459	32.3	58.7
"	26, "	1482	1478	7410	5.0	0	548	192	35.0	409	27.3	50.8
Sept.	2, "	1468	1453	8899	6.1	0	552	182	32.9	439	29.9	50.4
"	9, "	1228	1201	7228	6.0	0	418	134	32.0	444	36.1	52.5
"	16, "	581	488	4714	9.6	0	194	44	22.6	199	34.2	49.9
"	23, "	1421	1421	15339	10.7	0	516	141	27.3	463	32.5	60.9
"	30, "	939	936	10855	11.5	0	321	89	27.7	371	39.6	60.7
Oct.	7, "	828	825	9342	11.3	0	241	67	27.8	252	34.6	62.0
"	14, "	1432	1429	21882	15.3	0	502	95	18.9	456	31.8	59.6
"	21, "	1217	1215	20852	17.1	0	412	81	19.6	413	33.9	50.7
"	28, "	1125	1124	15906	14.1	0	382	75	19.6	356	31.6	47.1
Nov.	4, "	1082	1082	16195	14.9	0	397	64	16.1	297	27.4	45.1
"	11, "	912	911	11180	12.2	2	310	58	18.7	279	30.6	45.9
"	18, "	1155	1155	13980	12.1	10	401	55	13.7	365	31.6	48.2
"	25, "	1151	1151	13661	11.8	54	403	57	14.1	306	26.5	50.2
Dec.	2, "	906	906	13346	14.7	16	326	32	9.8	240	26.4	45.3
"	9, "	616	616	6544	10.6	50	220	35	15.9	120	19.5	51.1
"	16, "	628	628	5654	9.0	25	256	50	19.5	110	17.5	39.1
"	23, "	1102	1102	12006	10.9	93	418	55	13.2	204	18.5	45.9
"	30, "	838	835	10053	12.0	45	338	62	18.3	114	13.6	41.9
Jan.	6, 1912	673	673	6483	9.6	28	289	66	22.8	77	11.4	42.1
"	13, "	997	996	10022	10.1	121	451	88	19.5	128	12.8	41.7
"	20, "	993	992	11539	11.6	84	447	97	21.7	107	10.7	41.3
"	27, "	935	935	10838	11.6	172	376	96	25.5	143	15.3	46.9
Feb.	3, "	795	790	9477	12.0	190	361	92	25.4	119	15.0	33.3
"	10, "	618	618	8013	13.0	64	252	63	25.0	99	16.0	30.9
"	17, "	681	681	5774	8.5	51	274	89	32.5	135	19.8	34.2
"	24, "	949	949	9201	9.7	49	356	95	26.7	252	26.6	39.5

of the 1910-11 epidemic is noteworthy; about this time the largest percentage of adult pregnant female rats was found and, following this increase in breeding, the proportion of young to old rats rose from about 23 for every 100 to 40 for every 100.

These figures therefore seem to show:

(1) that a comparatively severe epidemic of plague, such as that which occurred in Cawnpore 1910-11, when the death rate from plague among men was 11.4 per mille, was associated with a marked reduction in the rat population.

(2) that this reduction in the rat population was followed by an increase in the proportion of female rats found pregnant, so that the rat population gradually increased again as the plague declined while the proportion of the young to old rats was at the same time raised.

(3) that as the rat population increased to a maximum the proportion of females which were pregnant decreased and this was followed by a fall in the relative number of young to old rats.

(4) with the advent of a fresh plague epidemic the rat population again decreased and the compensating arrangements for restoring the rat population to its normal standard were again in evidence.

These conclusions from our experience in Cawnpore must however be made with caution, for in Banda, where plague has only been of rare occurrence, we observed that even in the absence of a plague epidemic there is a well-marked seasonal variation in

(a) the number of rats caught in the houses.

(b) the proportion of female rats found to be pregnant.

(c) the proportion of young to old rats.

These seasonal variations, too, correspond very closely with those observed in Cawnpore (see under observations in Banda, p. 863).

*Fleas in Cawnpore.* Flea counts were made on more than fifty thousand rats during the year, the total number of fleas counted being 472,657, giving an average of about nine fleas per rat for the whole year. Almost all the fleas were *X. cheopis*, but 1112 were *Ceratophyllus fasciatus*, about 0.2 per cent. of the whole.

The number of fleas found on the rats varied from an average of 3.2 per rat in the end of June to 17.1 in the middle of October. During the winter months, December and January, there was a slight fall in the number of fleas found on rats but the number again increased in the spring. *Ceratophyllus fasciatus* was only found during the cold weather, the largest number being met with towards the end of January and beginning of February.

*The Immunity to Plague of Lucknow and Cawnpore Rats.*

We wish to refer the reader here to certain experiments, carried out during the course of this inquiry, which were designed to test the immunity to plague of rats caught in Lucknow and Cawnpore. The results of these experiments have been published in a paper which deals with the question of the immunity of the Indian wild rat to plague (see *Journal of Hygiene*, Plague Supplement II, January, 1913, pp. 229-65).

The summed results of comparative experiments with Lucknow, Cawnpore, Poona, Bombay, and Madras rats gave the following percentage mortality from plague in the respective groups.

Out of 500 Madras rats 485 died of plague	..	..	97 % mortality
Out of 598 Bombay rats 173 died of plague	..	..	29 % „
Out of 587 Poona rats 138 died of plague	..	..	24 % „
Out of 414 Cawnpore rats 75 died of plague	..	..	18 % „
Out of 349 Lucknow rats 117 died of plague	..	..	34 % „

These experiments showed that Cawnpore rats were at this time nearly twice as immune to plague as the Lucknow rats, and that the rats from these places were highly immune to the disease when compared with Madras rats which had not been exposed to infection. In the paper referred to above it was concluded that the relative immunity of rats was a rough measure of the persistence and severity of plague epidemics in the places in which they had been caught. It is possible that this relative immunity to plague is due to the fact that during successive epizootics, susceptible individuals die from the disease, and the more resistant animals that survive are able to transmit this characteristic to their young.

*The consideration of the question, Why has Cawnpore suffered more persistently and severely from plague than Lucknow?*

In attempting to answer this question we have shown that:

(a) Cawnpore is a much larger and more important trade centre than Lucknow, and having more intimate trade relations with many infected places in India it is exposed to greater risk of infection.

(b) Cawnpore is a more rat infested city than Lucknow so that when plague infection has been brought to the town the chance of the disease becoming permanently established is greater.



(c) Its extensive trade in grain, the construction of its houses, its dense population, its narrow streets, and the custom of its inhabitants, living as they do in close proximity to their grain stores and their domestic animals, bring a large section of the people into close association with rats which convey plague infection to them by means of infected fleas.

(d) The number of fleas found on the rats in Cawnpore is larger than in Lucknow and attains a maximum at an earlier period in the plague season, thus favouring the establishment of infection at an earlier date.

## CHAPTER IV

### OBSERVATIONS IN THE BALLIA DISTRICT

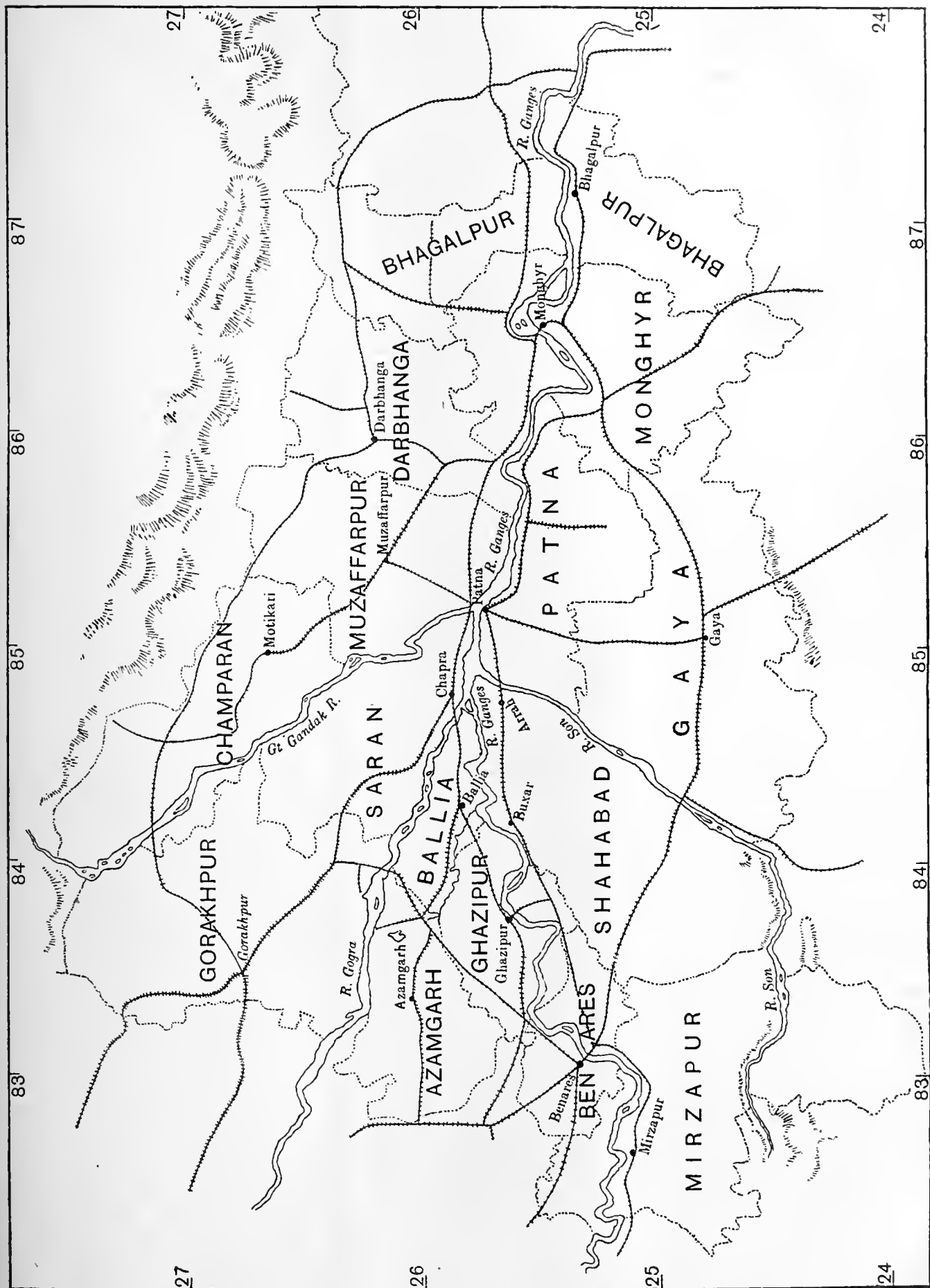
Having thus attempted to answer the first question we set out to solve, namely, why Lucknow city suffers less severely and less persistently from plague than Cawnpore, we now pass on to consider whether any explanation can be offered as to why the Ballia district has the unenviable reputation of being the most persistently and severely infected district in the United Provinces?

#### *Situation and Boundaries of the Ballia District.*

This district is the easternmost of the five which constitute the Benares division of the United Provinces (see Maps 1 and 2), and comprises an irregularly-shaped tract of country extending westwards from near the confluence of the Ganges and Gogra. The former river bounds it on the south, separating Ballia from the Bihar district of Shahabad, while the Gogra flows along the northern and eastern borders; the country beyond this river includes the Gorakhpur district of the United Provinces and the Bihar district of Saran. On the west, the boundary, except for the portion of the Sarju river, is for the most part artificial. Here Ballia is contiguous with Azamgarh on the north, and with Ghazipur on the south.

The extreme length of the district from east to west is about 63 miles, and the greatest breadth from north to south about 42 miles. The district does not extend to the actual confluence of the two great rivers





Map 2. BALLIA, with surrounding districts, showing Rivers and Railways.

# 834 *Epidemiological Observations in United Provinces*

but stops at the boundary of Shitab Diara, a mahal of Shahabad (see Map 3). The total area of the district varies from year to year and to a greater extent than that of any other district of the Provinces. This is due to erratic action of the Ganges and Gogra which are apt to vary their channels from time to time.

We may here draw attention to the fact that the Ballia district occupies the centre of a badly plague-infected area which surrounds the confluence of several great rivers; the Ganges, the Gogra, the Gandak and the Son (see Map 2).

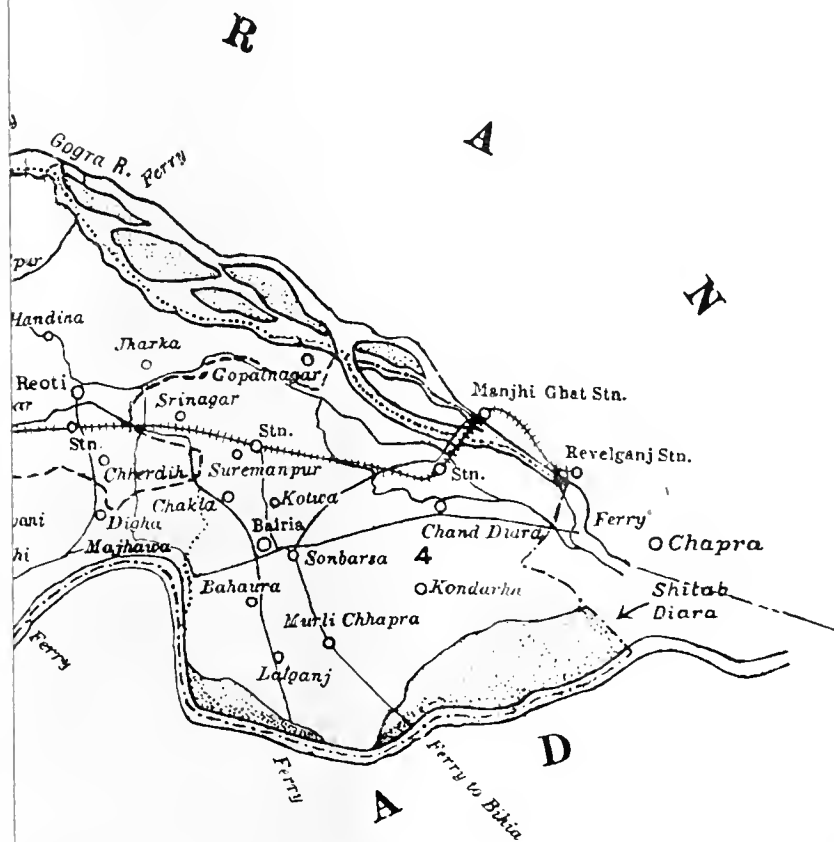
The area of severe infection, see Table XX, includes the whole of the Ballia district, the eastern portions of the Ghazipur and Azamgarh districts, the southern portions of the Gorakhpur district, almost the whole of the Saran district in Bihar and the northern part of the Shahabad district of the same Province which adjoins the Ballia and

TABLE XX. *Table showing number of deaths and deaths per mille in the plague-infected*

District	Population 1901	January to July 1900		1900—1		1901—2		1902—3		1903—4		1904—5	
		Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille
Patna	1624742	18408	11·3	31400	19·3	2214	1·4	8992	5·5	25103	15·4	27054	16·6
Gaya	2059933	5	—	11875	5·76	134	0·06	721	0·35	6220	3·0	18901	9·1
Shahabad	1962696	2	—	4468	2·3	2018	1·02	6579	3·5	10412	5·3	17812	9·0
Saran	2408814	1786	0·7	17958	7·4	9721	4·0	28694	11·9	13042	5·4	40854	17·0
Champanan	1790463	nil	—	nil	—	nil	—	17	0·01	nil	—	21	0·01
Muzaffarpur	2756130	17	—	1266	0·45	626	0·2	3294	1·2	2423	0·89	4680	1·7
Darbhanga	2912611	36	—	2362	0·8	2790	0·9	4301	1·5	1091	0·37	3321	1·14
Monghyr	2068804	1120	0·5	5665	2·7	970	0·4	4458	2·2	2932	1·4	11001	5·3
Bhagalpur	2088953	3	—	51	0·02	nil	—	220	0·1	1628	0·77	3412	1·5
Gorakhpur	2957074	—	—	2	—	2580	0·87	4225	1·42	8163	2·78	7343	2·50
Basti	1846153	—	—	—	—	8	—	1195	0·51	1674	0·91	4279	2·32
Azamgarh	1529785	—	—	—	—	508	0·33	3145	2·0	17407	11·24	17456	11·27
Ghazipur	913818	—	—	5	—	23	0·02	556	0·6	4985	5·45	28230	30·90
Ballia	937768	—	—	4181	4·2	10070	10·20	6286	6·36	14101	14·28	21037	21·29
Benares	882084	—	—	3042	3·4	129	0·14	2712	3·07	957	1·09	8695	9·86
Mirzapur	1082430	—	—	28	0·02	1857	1·7	129	0·1	1480	1·37	8085	7·47
Jaunpur	1202920	—	—	431	0·3	4916	4·08	5713	4·9	4119	3·42	12919	17·39

# District BALLIA

10 5 0 10 Miles.

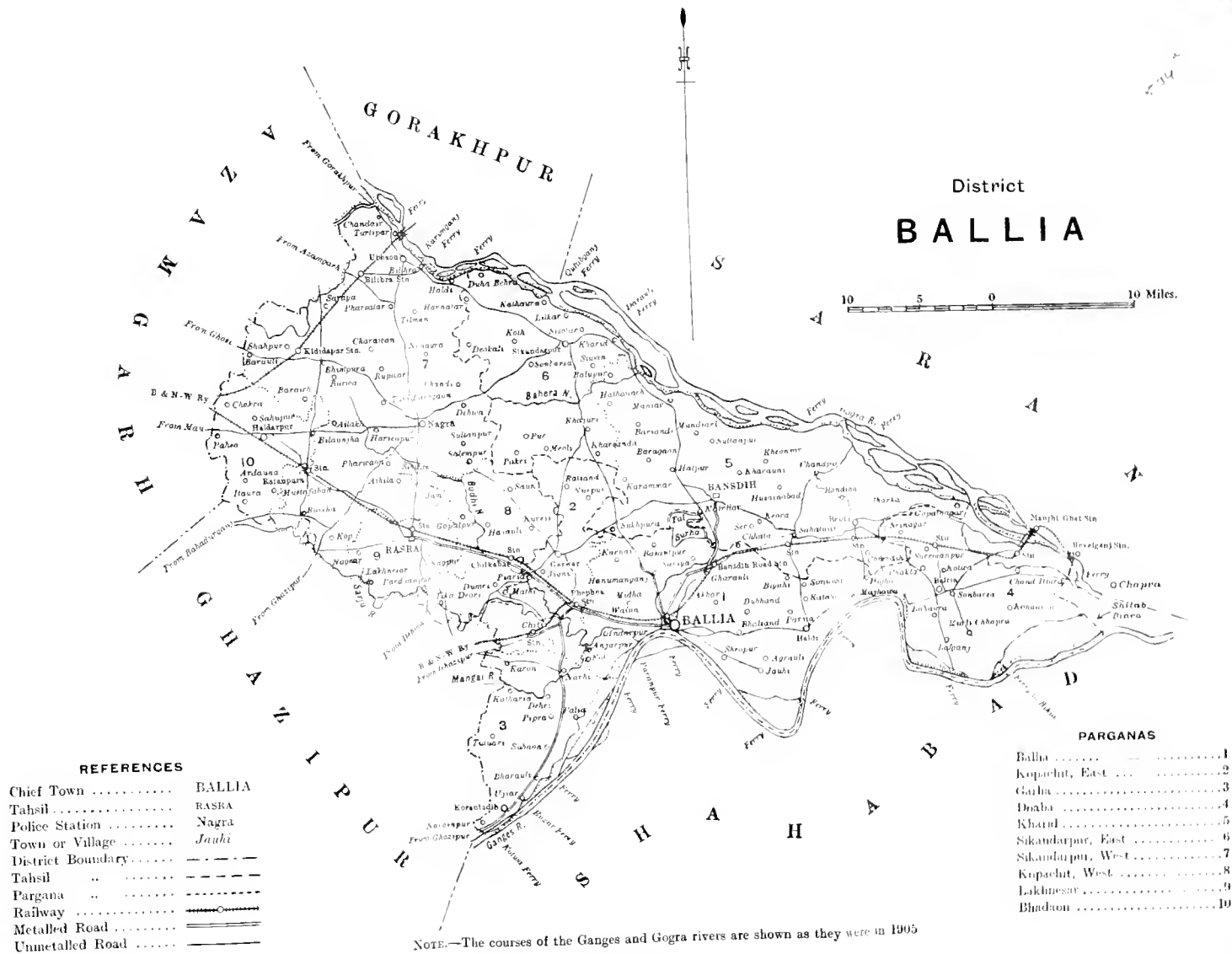


## PARGANAS

Ballia .....	1
Kopachit, East .....	2
Garha .....	3
Doaba .....	4
Kharid .....	5
Sikandarpur, East .....	6
Sikandarpur, West .....	7
Kopachit, West .....	8
Lakhnesar .....	9
Bhadaon .....	10

Chief  
Tahsil  
Police  
Town  
District  
Tahsil  
Pargan  
Railwa  
Metalle  
Unmet

were in 1905



Map 3.

Ghazipur districts of the United Provinces. The area of infection also includes the Patna district and parts of the Gaya, Monghyr and Bhagalpur districts of Bihar which lie along the Ganges.

*Physical Geography of the Ballia District.*

In its general aspects the district is a level plain without any hills or natural eminences. The only two natural divisions are those of the interior uplands which consist of comparatively old formation of alluvial deposit, and the more recent river alluvium which is constantly liable to change; the greater part of this is inundated during the rains.

The uplands have an average height of 210 feet above the sea level and roughly comprise the western half of the district. The

*districts in Bihar and adjoining districts of United Provinces, January 1900 to 1911.*

1905—6		1906—7		1907—8		1908—9		1909—10		1910—11		Total	Annual mean death rate per mille for 12 years 1900 to 1911
Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille	Total deaths	Deaths per mille		
14059	8.7	23461	14.4	2298	1.4	958	0.6	6936	4.2	—	—	163251	9.1
4880	2.4	7664	3.2	571	0.27	139	0.07	325	0.16	—	—	51666	2.3
5796	3.0	9817	5.0	2721	1.4	542	0.3	5674	2.9	—	—	67862	3.1
15039	6.2	31043	12.9	2915	1.2	1238	0.5	21507	9.0	—	—	185984	7.0
269	0.15	98	0.05	nil	—	nil	—	91	0.05	—	—	608	0.3
1817	0.65	4221	1.5	2173	0.8	369	0.13	538	0.2	—	—	21518	0.7
7256	2.5	2430	0.8	1877	0.6	1018	0.35	1488	0.5	—	—	28231	0.8
5600	2.7	2616	1.2	2491	1.2	1637	0.8	4704	2.3	—	—	44303	1.9
1354	0.65	142	0.07	826	0.4	136	0.06	31	0.01	—	—	7877	0.3
4837	1.64	7555	2.57	2865	0.97	700	0.24	17363	5.87	8319	2.60	63952	1.98
1864	1.01	1695	0.92	14	—	4	—	224	0.12	5514	3.01	16471	0.81
6777	4.38	12712	8.21	1655	1.08	1427	0.92	24766	16.19	22951	15.35	108804	6.40
884	0.97	13127	14.36	1607	1.76	553	0.61	6629	7.25	15582	18.31	72181	6.08
5506	5.57	22662	22.94	2627	2.66	4427	4.48	33403	33.01	11534	13.51	135834	12.50
722	0.82	1574	1.78	137	0.16	—	—	454	0.51	3470	3.87	21892	2.26
473	0.44	5373	4.97	107	0.10	—	—	398	0.37	7784	7.27	25714	2.16
789	0.65	2718	2.26	212	0.18	364	0.30	3644	3.03	19549	12.56	55374	4.18

lowlands comprise the eastern half of the district; most of this area, more especially the Paragana Doaba in the extreme east of the district, is liable to be submerged during the rains.

*Area and Population of the District.*

In 1906 the total area of the district was 793,623 acres as compared with 800,124 acres in 1901.

At the census of March 1901 it was ascertained that the inhabitants of the district numbered 987,366, showing a decrease of 7559 during the preceding ten years, but at the census in 1911 the number of inhabitants had fallen to 853,745, a loss during the decade of 133,621 or 13.5 per cent. This decrease has been largely due to severity of plague in this district.

Though there is no town of any considerable size in the district, the urban population is comparatively large, amounting to 11.3 %. In 1901 Ballia contained 1797 towns and villages and of this number 1566 had a population less than 1000; 157 others less than 2000, while of the remainder 55 possessed between 2000 and 5000 inhabitants and 19 more than 5000. The size of the villages in the Ballia district is remarkable, for excluding the chief towns they average 491 inhabitants each, the corresponding figure for Ghazipur being 399. This difference is far greater still if only the eastern portions of the district are considered, where the average population of a village in the Ballia paragana is 508 and in the Doaba 912. The large number of comparatively large villages in this district must be a factor of considerable importance in the spread of plague. An additional factor of some importance in the spread of plague is found in the fact, as revealed by the census enumeration, that, as in Ghazipur district, very large numbers of Ballia labourers resort to the industrial centres of Howrah, Calcutta and elsewhere.

*The Climate of the Ballia District.*

There was no meteorological observatory in the Ballia district till we erected, in Ballia town in April 1911, an observing station of the standard pattern adopted by the Indian Meteorological Department. Records of rainfall are available, however, for many years. Rain gauges are maintained at each of the tahsil headquarters.

The average annual rainfall for the whole district for 42 years has been 41.82 inches. The corresponding average for the 10 years 1901 to

1911 is 39·4. Table XXI gives the actual monthly rainfall in the district (based on the average of the three recording stations) from July 1901 to June 1911.

TABLE XXI. *Rainfall in Ballia District, 1901 to 1911.*

Years	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Yearly totals
1901-02	11·03	10·50	5·05	0·26	0·20	—	—	0·12	0·37	—	1·32	2·15	30·98
1902-03	10·22	8·31	14·65	0·10	0·03	—	—	0·13	0·07	—	—	5·61	39·12
1903-04	3·72	14·89	6·20	8·37	—	—	0·40	0·13	—	—	1·03	7·00	41·74
1904-05	17·47	11·36	1·42	5·05	0·12	0·32	0·30	0·39	1·11	0·10	1·29	0·45	39·38
1905-06	16·64	13·57	11·64	0·49	—	0·04	0·26	3·12	0·28	—	0·01	2·62	48·67
1906-07	11·26	14·88	2·37	1·19	—	—	0·04	4·55	0·55	0·59	0·01	4·16	39·57
1907-08	5·30	6·32	4·90	0·08	—	—	0·80	0·99	0·12	—	0·02	3·98	22·51
1908-09	5·61	11·87	—	—	—	—	0·08	0·47	—	2·03	0·02	11·04	31·12
1909-10	12·80	12·64	4·31	1·23	—	1·00	0·07	0·01	0·07	0·14	0·62	5·19	38·08
1910-11	8·56	17·22	9·71	5·07	0·92	—	0·41	—	1·39	0·17	1·55	7·85	52·85
Av. for years													
1901-11	10·26	12·15	6·02	2·19	0·127	0·136	0·236	0·991	0·396	0·303	0·587	5·00	39·4

An examination of this table shows that the years 1904-5, 1906-7, 1909-10 were characterised by the fact that some rain fell in every month from January to June. A reference to Table XXIII will show that these have been the years when plague has been most severe in this district. By contrast, the years 1907-8 and 1908-9 were dry years and these, it will be seen, were years of mild plague epidemics. See Chart VIII (below, p. 879).

Records of temperature and humidity at Ballia are available from April 1911, when the observing station was established by the Commission in this town. For purposes of contrast, Table XXII gives the monthly means of temperature and relative humidity for Ballia, Gorakhpur, Benares, Agra, Cawnpore, Lucknow and Jhansi for the period from April 1911 to May 1912.

It will be seen that the monthly means of the mean between the maximum and minimum temperatures, recorded in Ballia, lie between those of Benares and Gorakhpur. The 8 a.m. humidity during April, May and June was higher in Ballia than in either Benares or Gorakhpur, but for the remainder of the period the humidity in Ballia lay between those of the other two stations. It will also be seen that, during the hot weather, the temperature is lower and the humidity higher in Ballia than in either Agra, Cawnpore, Lucknow or Jhansi; these are conditions which are relatively favourable for the spread of plague.

TABLE XXII. *Monthly Mean of Mean between Maximum and Minimum Temperatures.*

Stations	1911										1912				
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	
Ballia	85.2	90.2	87.1	89.6	83.3	82.7	78.8	68.1	59.6	61.0	67.3	75.1	85.5	90.1	
Gorakhpur	83.5	88.5	86.9	87.2	82.1	82.0	78.1	67.3	59.6	60.4	66.1	73.0	81.2	86.4	
Benares	85.4	94.2	89.6	90.0	83.7	83.1	79.5	68.8	59.6	61.6	68.6	75.0	86.6	92.8	
Agra	86.5	96.9	96.3	95.6	91.8	90.6	82.0	67.9	61.3	61.5	67.2	74.2	—	—	
Cawnpore	85.9	94.7	94.5	93.8	86.7	81.8	79.6	67.3	59.9	61.5	67.3	74.3	86.4	92.9	
Lucknow	85.7	94.0	92.2	93.5	84.6	81.7	79.5	67.2	59.2	61.1	66.9	73.9	84.9	91.1	
Jhansi	88.0	98.1	93.6	92.4	87.3	80.8	80.0	68.7	62.3	64.0	69.7	76.0	88.5	96.4	

*Monthly Means of 8 a.m. Humidity.*

Ballia	49	64	75	72	90	88	84	80	84	89	72	48	66	63
Gorakhpur	46	61	74	81	91	90	86	83	87	92	79	53	65	62
Benares	42	49	71	66	88	88	80	82	80	87	70	43	50	47
Agra	32	28	53	55	63	83	65	67	64	80	67	40	—	—
Cawnpore	31	36	53	58	78	89	77	75	78	85	72	43	43	41
Lucknow	36	54	61	59	85	88	77	88	74	85	67	42	48	47
Jhansi	24	23	57	63	68	82	67	70	66	77	56	25	26	24

TABLE XXIII. *Monthly statement of Deaths from Plague in the Ballia District. (Includes town and rural circles.)*

	1900-1	1901-2	1902-3	1903-4	1904-5	1905-6	1906-7	1907-8	1908-9	1909-10	1910-11
July	—	—	9	—	141	4	24	41	1	340	15
August	—	—	32	3	507	36	161	86	—	823	5
September	—	40	81	1	397	26	201	59	3	899	2
October	—	124	192	91	441	91	155	21	—	930	17
November	—	148	523	392	789	109	714	99	75	2483	172
December	—	785	809	991	2519	420	1650	359	273	5804	656
January	—	2303	978	2089	3595	628	3004	378	526	8076	1150
February	310	2343	955	3381	3537	949	4350	576	652	6574	1886
March	2150	2799	1598	4977	5456	1880	7353	696	1311	5774	3991
April	1428	1150	941	1907	2912	1215	3783	302	754	1428	3012
May	286	307	151	224	680	145	1167	10	691	227	606
June	7	71	17	45	63	3	100	—	141	25	22
Totals	4181	10070	6286	14101	21037	5506	22662	2627	4427	33403	11534
Per mille	4.2	10.2	6.36	14.28	21.29	5.57	22.94	2.66	4.48	33.81	11.7

*Communications in the District.*

The roads are bad, with the exception of the few which have been metalled, and considerable difficulty is experienced in conveying merchandise from the villages to the chief trade centres. This is illustrated



by the small extent to which carts are used in Ballia as compared with the western districts, most of the local trade being carried by means of pack bullocks and ponies. In spite of these drawbacks, however, the district possesses very fair commercial facilities, for, at the present time, it is supplied with 89 miles of railway in addition to 120 miles of river frontage.

The northern portion of the district is for the most part still remote from the railways.

#### *Agriculture.*

The chief autumn (*kharif*) crops are sugar-cane, rice, maize and various millets and pulses.

Of the spring (*rabi*) crops, barley, wheat, gram, peas and linseed are the most important. The chief feature of the district is fertility. This is specially so in the Doaba. Irrigation, when required, is carried out chiefly from wells. The depth at which water is found averages about 15 ft. in the alluvial tracts and 20 ft. in the uplands. The district has been almost free from famine.

#### *Trade.*

The trade consists chiefly of agricultural products. Imports of food-stuff consist principally of rice from lower Bengal and Gorakhpur. This may come in at any time of the year. The exports are chiefly sugar, gram, barley, wheat, oil-seeds and peas. Much of this goes to Bihar and Bengal.

#### *Fairs and Pilgrimages.*

The fairs are religious in origin, and in many instances the celebration of some festival is the main object of the assemblage. The large fairs, however, now derive their importance from their commercial aspect. By far the most important fair held in the district is the Dadri Mela which takes place at Ballia on the full moon of November and attracts some 500,000 persons. The attendance has doubled during the last twenty years and there has been a proportionate increase in the volume of trade. The fair is held at the site of the former confluence of the Sarju with the Ganges, which took place at some distance to the east of Ballia town. The increase in size and importance of the gathering has necessitated elaborate sanitary and police arrangements. Two large enclosures are provided for cattle and horses while shops of all sorts and descriptions are put up in regularly laid out streets. In 1902 there

were 1372 shops in which articles of every description were exposed for sale.

Few of the other fairs are of importance; the largest is held at Raniganj in Bairia Circle and lasts for ten days, the attendance on the principal day being about 20,000.

#### *Houses.*

The great majority of the houses in the villages are built of mud and have tiled or thatched roofs. These houses are closely built, but are not crowded together to the same extent as in the compact villages of the Punjab. A few inhabited sites, in parts of the district, are liable to inundation, especially in the Paragana Doaba. The houses on these sites consist of flimsy grass huts which are removed or abandoned on the onset of the rains.

#### *History of Plague in Ballia District.*

*First outbreak of Plague.* Plague spread from Calcutta to the western districts of Bengal (now Bihar) in the cold weather of 1898-99. The Bihar districts of Patna and Saran (see Map 2 and Table XX) were, from the first, severely infected. Shahabad, the other district of Bihar adjoining Ballia, was not infected till January 1901. It was early recognised that the risk of infection being brought into Ballia district from Saran was very great, as free communication exists between the eastern part of this district and the towns of Chapra and Revelganj in Saran. The latter town is just across the Gogra river and its markets supply Kotwa and other bazaars in the Doaba with grain. An elaborate system for the medical inspection of travellers visiting the district was therefore devised and the arrangements were placed under the supervision of a medical officer and the Collector of the district.

During the cold weather of 1899-1900 the district escaped infection. Indications were, however, not wanting in the following autumn that there would be a virulent outbreak of the disease in the western districts of Bihar during the ensuing cold weather, and so more effective supervision was enjoined. From November 1900 onward refugees from the infected districts of Bihar began to pour into the Ballia district by rail, road and river. During November, December and January the number of arrivals from infected districts was 11,500.

The first report of plague within the district was received from Raniganj, a market town in Bairia Police Circle in the east of the

district. The earlier deaths from plague were not discovered at the time they occurred but subsequent inquiry showed that the first death took place on the eleventh of January in Raniganj.

Three suspicious deaths, immediately following the first, were discovered to have occurred in the house of a grain dealer. His house immediately adjoined that in which the first case was found. This grain dealer had large dealings in grain with Revelganj which was then infected<sup>1</sup>.

By the end of February reports of infection had come to hand from thirteen villages and one large town, Sahatwar (population 11,500), and the total number of deaths was 320. By the end of March fifty-three villages had become infected. The greatest mortality occurred in the last week of March when 645 deaths were reported. During April there was a distinct falling off in the severity of the disease and by the end of May the epidemic had practically terminated. In June only seven deaths were reported—the last on the 27th June. The total number of deaths reported during the epidemic was 4181 and the number of infected villages was 84.

*The second epidemic.* During the months of July and August 1901 no cases of plague were reported in the district. Early in September, however, the disease broke out in Bansdih, a large town about ten miles north of Ballia, which had reported one death from plague in the previous April.

Plague appeared, about the middle of September, in Bhelsand, a large village six miles west of Ballia, and in Suremanpur and Murarpatti, two villages in the Doaba. Bhelsand had reported twenty-one deaths in the previous epidemic, the last on May 14th; Suremanpur ten deaths, the last on May 19th, and Murarpatti two deaths on May 4th.

The epidemic spread over the whole district; the highest mortality was in Ballia and Ubhaon Circles.

*Subsequent epidemics.* The numbers of deaths which have occurred in all the epidemics of plague in the Ballia district up to the year ending June 1911 are given in Table XXIII. It will be seen that very severe epidemics of plague occurred in the years 1904–5, 1906–7, and 1909–10, and that comparatively mild epidemics prevailed in the years 1907–8, and 1908–9. We have already noted the unusually heavy winter and

<sup>1</sup> It is interesting to note that in the report on the first epidemic which was submitted by the Collector and the Civil Surgeon, considerable stress is laid on the importance of grain as a means of disseminating infection. In fact the rapidity of the spread of the disease from Raniganj is attributed to the fact that this town contains a large grain market which supplies numerous villages round about.

spring rainfall of the former years but we must leave for subsequent discussion the other factors which may have favoured or prevented the spread of plague throughout the period under observation.

*Observations on Rats and Fleas in Ballia.*

The methods adopted for the examination of rats and fleas were similar to those which we employed in Lucknow and Cawnpore. The numbers of rats of different kinds caught from the 23rd January 1911 to the 31st May 1912, were:

<i>Mus rattus</i>	12,054
<i>Gunomys varius</i>	14
Musk rats	2894
Mice	757

In Ballia district, during the plague season, we were unable to secure a continuous supply of rats sufficient for our purpose in any one place. This necessitated frequent transfers of our staff and the extension of trapping to a large number of villages and towns. For this reason we have not attempted to tabulate our results as we have in the case of Cawnpore and Lucknow but will simply state, in the form of general conclusions, our experience of rat catching in the Ballia district.

We found that the appearance of plague in a town or village was quickly followed by a reduction in the number of rats that could be trapped. For example: when we commenced trapping in Ballia town during the last week of January the average number of rats caught varied from 57 to 36 per 100 traps set, which indicated a relatively high degree of rat infestation. At this time plague was not severe, only two to three deaths a week being reported. A marked increase in the severity of the epidemic occurred during the third week of February and simultaneously the rats dropped to under 10 per 100 traps set.

Again Bairia town was suffering from a fairly severe epidemic when we began trapping there in the last week of March, and our catches averaged only 12 rats per 100 traps set. At the same time we were able to catch from 25 to 35 rats per 100 traps in several villages adjoining Bairia which were free from plague.

Trapping was carried out continuously in Rasra town from August 1911 to February 1912. Up till December 1911 the town remained free from plague. During the plague-free period the number of rats per 100 traps varied from about 30 to 60. Plague broke out in Rasra

early in December and during this month the rats caught averaged only 20 per 100 traps. Coincidentally with an increase in the number of plague deaths, the number of rats fell still further to less than 12 per 100 traps during January and February.

TABLE XXIV. *Monthly figures showing the number of Mus rattus on which flea count was made, total fleas and average number of fleas per Mus rattus in Ballia.*

Month	No. of <i>Mus rattus</i>	Total fleas	Average no. of fleas per <i>Mus rattus</i>
January 1911	160	1403	8.7
February „	481	3772	7.9
March „	478	4771	9.9
April „	382	3908	10.2
May „	—	—	—
June „	108	673	6.2
July „	316	1775	5.6
August „	2483	11895	4.8
September „	2739	22607	8.3
October „	1382	12621	9.1
November „	1068	10578	9.9
December „	726	7954	11.0
January 1912	392	4098	10.5
February „	248	2409	9.7
March „	103	1618	15.7
April „	385	1795	4.7
May „	103	690	6.7

TABLE XXV. *Showing the monthly prevalence of fleas on Mus rattus in Ballia, Lucknow and Cawnpore.*

Month	No. of fleas per <i>Mus rattus</i>		
	Ballia	Lucknow	Cawnpore
January 1911	8.7	12.5	—
February „	7.9	11.6	14.7
March „	9.9	11.7	14.2
April „	10.2	10.2	13.4
May „	6.7 <sup>1</sup>	4.8	6.0
June „	6.2	2.6	3.4
July „	5.6	3.4	3.2
August „	4.8	2.4	4.7
September „	8.3	3.5	9.1
October „	9.1	9.6	14.7
November „	9.9	10.7	13.1
December „	11.0	8.5	10.8
January 1912	10.5	11.8	10.8

<sup>1</sup> Is the figure for 1912; figure for 1911 is not available.

For similar reasons to those already given we have not tabulated the figures showing the number of pregnant females or young rats

caught in different months of the year; breeding appeared to be most active during the hot weather and rains (April to September), and young rats least numerous in January and February, following a period of diminished fertility in November and December.

With the exception of a few cat fleas, the only kind of flea found on the rats in Ballia district was *Xenopsylla cheopis*. Table XXIV gives the monthly prevalence of this flea on *Mus rattus*. It will be seen that, except in March 1912, the average number of fleas per rat did not exceed eleven. In March 1912 most of the flea counts were made in the town of Chit Baragaon which was severely infected with plague at the time. On fifty-two rats caught in that town the fleas averaged twenty-one per rat. The March figure therefore must be regarded as exceptional.

On the other hand, the flea infestation of the rats during the non-epidemic season—June to August—was distinctly greater in Ballia than in Cawnpore or Lucknow. This is shown in Table XXV in which the flea prevalence in the three places is compared. We shall have occasion to refer to this high prevalence of fleas in the off-season when discussing the question of the persistence of plague in Ballia district.

*The Immunity of Ballia rats to Plague.* The degree of immunity to plague of Ballia rats was tested by the method which is described in detail elsewhere in these reports (*Journal of Hygiene* (Supplement), 1913, page 229 et seq.). Briefly this consists in inoculating a series of Madras rats, which possess very little immunity, with a dose of plague bacilli just short of that required to kill 100 per cent., and at the same time inoculating the rats whose immunity it is desired to test with the same dose. The percentage of rats which survive the inoculation is taken as a measure of the immunity of each series.

Two experiments with Ballia rats showed that while 99 % of Madras rats died of plague with the dose used, only 38 % of rats from Chit Baragaon and 37 % of rats from Bansdih perished. Chit Baragaon and Bansdih are large villages in Ballia district. These figures may be compared with those obtained in the same experiments for rats from Cawnpore and Lucknow which had respectively a mortality of 19 % and 32 %. Both places in Ballia, from which rats were obtained, had suffered severely from plague, deaths among the inhabitants have occurred in them almost every year from 1901 to 1911, totalling, in the case of Chit Baragaon 1872 or an average death rate per mille per annum of 19.7, and, in the case of Bansdih, 1710 deaths or 17 per mille per annum. It will be noted that despite the severity of plague in these

villages the rats were more susceptible to the disease than those from either Cawnpore or Lucknow, although they were relatively immune compared with Madras rats.

*Consideration of the Factors which may Account for the Severity of Plague in Ballia District.*

Plague in the Ballia district has been characterised by persistence from year to year, rather than by the outstanding severity of any particular epidemic or epidemics. It is true that in 1909-10 the deaths from plague reached the high figure of 33,402, representing a mortality of about 36 per 1000 of the estimated population. This figure has however been far exceeded in the severest epidemics in Muttra and Muzaffarnagar and in many of the epidemics of the badly infected districts of the Punjab.

On the other hand, during the years when plague in the United Provinces as a whole has been of minimum severity, the mortality in Ballia has been considerable; never less than 2.6 per 1000 population.

Closely related with the severity of plague in Ballia is the persistence of the disease through the non-epidemic season. Since the disease broke out in January 1901 deaths from plague have occurred during every month except July and August 1901, July 1903 and June, August and October 1908. A similar persistence through the off-season is found in districts adjoining Ballia. For instance, in Azamgarh plague deaths have been reported every month since the district first became infected in January 1902, and the Bengal district of Saran which first became infected in January 1899 has been free for only three months—July to September 1908.

In Table XXVI the extent to which plague is carried over from one epidemic season to another in different districts of the United Provinces is shown. It will be seen that 57 % of all the plague deaths in the United Provinces during July and August (1901-10) were reported from the south-eastern districts, Gorakhpur, Azamgarh, Ghazipur and Ballia, while Ballia district alone contributed 26 % or about one-quarter of the total deaths from plague during these months.

A list of the villages and towns which reported deaths from plague in June, July and August 1911 is given in Table XXVII. The monthly mortality from plague in each of these villages is shown during the plague season 1910-11 and up to January 1912. It will be seen that in June ten villages reported twenty deaths, in July twenty-four villages seventy-six deaths, and in August nine villages twenty deaths. Three



TABLE XXVI (United Provinces). Table showing percentage of total deaths from plague in July and August in the provinces in each district.

846 *Epidemiological Observations in United Provinces*

	1901		1902		1903		1904		1905		1906		1907		1908		1909		1910		Total deaths reported in July and August of all years	Percentage of provincial totals 1901 to 1910
	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total	Total deaths	% of provincial total		
Dehra Dun	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	0.2
Saharanpur	—	—	2	0.3	1	1.7	82	3.5	1	—	13	1.6	22	3.3	—	—	—	—	—	—	121	1.4
Muzaffarnagar	—	—	—	—	—	—	43	1.8	16	—	4	0	20	3	2	2.1	—	—	1	—	86	1.0
Meerut	—	—	—	—	1	1.7	6	—	5	—	—	—	21	3	2	2.1	—	—	5	—	40	0.5
Bulandshahr	—	—	—	—	—	—	—	—	2	—	—	—	8	1.2	—	—	—	—	3	—	14	0.2
Aligarh	—	—	—	—	—	—	20	9	68	8.2	—	—	23	3.4	15	16	—	—	4	—	88	1.0
Muttra	—	—	—	—	—	—	19	8	75	9	—	—	—	—	—	—	—	—	—	—	136	1.5
Agra	—	—	—	—	—	—	3	—	1	—	—	—	—	—	—	—	—	—	26	3.7	40	0.5
Farukhabad	—	—	—	—	—	—	38	1.6	4	—	2	—	22	3.3	—	—	—	—	1	—	84	0.9
Mainpuri	—	—	—	—	—	—	13	—	14	1.7	—	—	—	—	—	—	—	—	—	—	50	0.6
Etawah	—	—	—	—	—	—	9	—	14	1.7	1	—	—	—	2	2.1	—	—	19	2.7	82	0.9
Etah	—	—	—	—	—	—	—	—	22	2.7	—	—	14	2	2	2.1	—	—	4	—	50	0.6
Bareilly	—	—	—	—	—	—	14	—	9	1	1	—	4	—	—	—	—	—	1	—	44	0.5
Bijnor	—	—	—	—	—	—	11	—	33	4	14	1.7	26	3.9	3	3.2	—	—	0	—	87	1.0
Budaun	—	—	—	—	—	—	—	—	2	—	13	1.6	16	2.4	—	—	—	—	8	—	41	0.5
Moradabad	—	—	—	—	—	—	—	—	3	—	2	—	50	7.6	—	—	—	—	—	—	66	0.8
Shahjahanpur	—	—	—	—	—	—	—	—	1	—	3	—	10	1.5	—	—	—	—	—	—	17	0.2
Pilibhit	—	—	—	—	—	—	3	—	9	1	2	—	—	—	—	—	—	—	—	—	66	0.8
Cawnpore	—	—	—	—	—	—	16	—	9	1	43	5.3	22	3.3	—	—	—	—	93	13	104	1.2
Fatehpur	—	—	—	—	—	—	6	—	16	2	15	1.8	22	3.3	—	—	—	—	6	—	640	7.5
Banda	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	1.4	69	0.8
Hamirpur	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	0.1
Allahabad	4	100	110	16	22	37.5	97	4.2	92	11	6	—	10	1.5	1	1	—	—	1	—	343	4.0
Jhansi	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Jalaun	—	—	—	—	—	—	39	1.6	0	—	—	—	—	—	—	—	—	—	5	—	45	0.5
Benares	—	—	—	—	—	—	13	—	—	—	—	—	—	—	—	—	—	—	—	—	35	0.4
Mirzapur	—	—	—	—	—	—	79	3.4	—	—	14	1.7	8	1.2	—	—	—	—	—	—	128	1.5
Jaunpur	—	—	—	—	—	—	65	2.8	2	—	46	5.6	1	—	—	—	—	—	2	—	116	1.3
Ghazipur	—	—	—	—	—	—	607	26.3	5	—	1	—	3	—	12	12.8	—	—	17	2.4	769	9.0
Ballia	—	—	—	—	—	—	648	28	40	5	18	2.2	9	1.3	—	—	—	—	30	4.2	2228	26
Gorakhpur	—	—	—	—	—	—	78	3.4	142	17	185	22.6	127	19	1	1	—	—	20	3	946	11.0
Basti	—	—	—	—	—	—	29	1.3	4	—	40	5	142	21	47	50	—	—	93	13	285	3.2
Azamgarh	—	—	—	—	—	—	115	5	185	22.4	134	16.4	1	—	6	6.4	—	—	116	16.3	936	11.0
Naini Tal	—	—	—	—	—	—	—	—	—	—	88	10.8	48	7.2	—	—	—	—	50	7	—	—
Almora	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Garhwal	—	—	—	—	—	—	15	—	—	—	—	—	—	—	—	—	—	—	—	—	18	0.2
Lucknow	—	—	—	—	—	—	55	2.4	4	—	20	2.4	5	—	—	—	—	—	3	—	89	1.0
Unao	—	—	—	—	—	—	33	1.4	—	—	17	2	—	—	—	—	—	—	11	1.6	162	1.9
Rae Bareli	—	—	—	—	—	—	32	1.4	—	—	37	4.5	1	—	—	—	—	—	5	—	81	0.9
Sitapur	—	—	—	—	—	—	—	—	23	2.8	19	2.3	2	—	—	—	—	—	—	—	44	0.5
Hardoi	—	—	—	—	—	—	2	—	—	—	—	—	4	—	—	—	—	—	47	6.5	58	0.7
Kheri	—	—	—	—	—	—	—	—	—	—	4	—	2	—	—	—	—	—	—	—	6	0.07
Fyzabad	—	—	—	—	—	—	27	1.2	—	—	2	—	5	—	—	—	—	—	18	2.5	52	0.6
Gonda	—	—	—	—	—	—	13	—	—	—	21	2.6	1	—	—	—	—	—	82	11.5	117	1.3
Bahraich	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	2	—	17	0.2
Sultanpur	—	—	—	—	—	—	6	—	1	—	—	—	4	—	1	1	—	—	9	1.3	22	0.3
Partabgarh	—	—	—	—	—	—	1	—	17	2	12	1.5	—	—	—	—	—	—	1	—	41	0.5



TABLE XXVII. Villages in Ballia District which reported deaths from plague in June, July and August 1911.

Thana	Name of village	Date of first death in 1910-11	No. of deaths reported											
			Up to Mar. 1911	Mar. 1911	Apr. 1911	May 1911	June 1911	July 1911	Aug. 1911	Sept. 1911	Oct. 1911	Nov. 1911	Dec. 1911	Jan. 1912
Bansdih	Barsare	28. 12. 10	48	—	7	—	—	—	2	1	3	1	3	6
	Bansdih	4. 3. 11	—	3	30	33	5	5	—	2	3	14	52	97
	Bamnarli	30. 6. 11	—	—	—	—	1	6	—	—	—	—	—	16
	Baghauli	8. 7. 11	—	—	—	—	—	1	—	—	—	—	—	—
	Chainpur	25. 7. 11	—	—	—	—	—	2	—	—	—	—	—	—
Garwar	Kharauni	21. 8. 11	—	—	—	—	—	—	3	14	27	29	25	29
	Garwar	28. 3. 11	—	2	3	2	—	4	1	—	—	—	6	31
	Kopwa	7. 6. 11	—	—	—	—	1	4	—	—	—	—	—	—
	Sawan	10. 7. 11	—	—	—	—	—	2	—	—	—	—	—	—
	Sibpur	16. 7. 11	—	—	—	—	—	4	—	—	—	8	8	—
Bairia	Maniar	17. 8. 11	—	—	—	—	—	—	1	—	—	3	5	1
	Srinagar	20. 2. 11	—	2	8	8	1	1	—	—	—	—	1	46
	Karmanpur	22. 2. 11	—	1	—	13	2	2	—	—	8	13	6	—
	Sonbarsa	24. 3. 11	—	1	6	4	1	—	—	—	2	22	47	26
	Charajpur	9. 4. 11	—	—	3	—	—	2	—	—	—	—	5	9
Sikandarpur	Dokali	10. 4. 11	—	—	3	—	—	3	1	—	12	9	7	3
	Sawan Chhapra	17. 4. 11	—	—	2	—	1	—	—	—	—	—	4	3
	Milki Tiwari	17. 4. 11	—	—	1	5	3	—	—	—	—	3	25	28
	Tengarahi	18. 6. 11	—	—	—	—	3	1	5	4	17	2	7	1
	Chandpur	27. 8. 11	—	—	—	—	—	—	1	—	10	25	11	—
Haldi	Pur	5. 4. 11	—	—	11	7	—	5	—	—	—	—	—	7
	Koth	20. 4. 11	—	—	6	1	2	5	—	—	—	—	—	27
	Majanlia	1. 5. 11	—	—	—	8	—	1	—	—	—	—	5	8
	Bigahi	2. 7. 11	—	—	—	—	—	7	—	—	5	33	14	2
	Saluhar	2. 7. 11	—	—	—	—	—	2	—	—	—	—	—	12
Ballia	Reoti	2. 10. 10	22	39	85	12	—	—	—	—	1	—	—	3
	Rampur Tilahi	12. 2. 11	1	9	—	—	—	—	5	—	—	4	5	6
	Nagwa	24. 2. 11	1	1	—	—	—	6	—	3	34	29	5	6
	Bishimpura	3. 7. 11	—	—	—	—	—	8	—	—	—	10	16	17
	Arhat Doobyka Chhapra	18. 7. 11	—	—	—	—	—	1	1	—	—	8	4	9
Bhelsand	Barmain	19. 7. 11	—	—	—	—	—	2	—	1	2	5	5	11
	Bhelsand	15. 8. 11	—	—	—	—	—	1	—	7	13	53	21	1

villages were newly infected in June, ten in July and three in August. Many of these villages were visited by the Commission and members of the inoculating staff who saw undoubted cases of plague.

We were able to demonstrate the existence of acute plague among the rats in Bansdih Circle where rat trapping was being carried on in the off-season of 1911. In Bansdih town three plague-infected rats were found between the 13th July and 4th September. During this period only three or four plague deaths were reported in the town and a few cases of plague which recovered came under our observation.

In Kharauni, a large village near Bansdih, plague rats were found on the 28th July and the 3rd August. An epidemic followed, the first death being reported on the 21st August. An infected rat was found in Bakwa village in the Bansdih Circle on the 11th September, but no plague deaths were reported until January 1912.

The finding of rats with acute plague in Ballia during the non-epidemic season is in marked contrast with our experience in Lucknow and Cawnpore, where, although relatively large numbers of rats were examined, we found no evidence of an epizootic going on in the interval between the epidemics.

The persistence of plague throughout the non-epidemic season of 1911 may therefore be correlated with:

(1) The observed high prevalence of fleas in June, July and August to which we have already alluded (Tables XXIV and XXV).

(2) Our meteorological observations, which show that in May, June and July 1911, the relative humidity in Ballia was higher than that recorded at any other station in the United Provinces, while Rurki and Gorakhpur alone recorded lower monthly mean temperatures during this period.

These conditions of high humidity and low temperature in April, May and June, which are shared in a more or less degree by all the south-eastern districts, would, as far as we know, be favourable to the persistence of plague in the non-epidemic season. But the relation between high humidity and low temperature and plague mortality is by no means constant. Thus the Bahraich district which is particularly moist and cool (see Table I) suffers only little from plague, and Darbhanga in Bihar, which is more humid and cooler than Ballia or the adjacent districts of Patna, has suffered hardly at all. In these districts, however, other factors may possibly play a part in counteracting the favourable climatic conditions for plague prevalence.

These considerations show how difficult it is to weigh the relative

importance of the climatic factor. Although we can find no constant relation between the climatic conditions and plague mortality in one district as compared with another, we have shown that within a given district the relative humidity during the epidemic season appears to influence the mortality, a high humidity during the winter months being associated with severe plague.

The greater density of the population in the Ballia district may also be favourable to the diffusion of the disease.

A reference, however, to the following Table (XXVIII) shows that there is not of necessity any direct relation between density of population and plague mortality even in the case of districts in the same province. In some cases, this lack of correlation is more apparent than real. In the Shahpur district of the Punjab, for example, where the table shows the density of population to be 108 per square mile, *i.e.* about one-seventh that of Ballia, the death rate from plague is 14·1 higher than that of Ballia. The population of this district is, however, concentrated in the irrigated valleys of the Jhelum and Chenab, where it is almost as dense as that of Ballia, while a large portion of the district is very sparsely populated.

TABLE XXVIII.

Province	District	Density of population per sq. mile	Average annual mortality from plague per 1000 population (1900—1911)
The Punjab	Ludhiana	462	22·3
	Gujranwala	293	17·9
	Shahpur	108	14·1
	Amritsar	639	11·5
United Provinces	Ballia	792	12·5
	Benares	874	2·2
	Muzaffarnagar	531	11·2
Bihar	Saran	901	7·0
	Muzaffarpur	908	0·7
	Darbhangha	870	0·8

## CHAPTER V

## OBSERVATIONS IN THE BUNDELKHAND

We may pass on now to consider the third problem we attempted to solve in our epidemiological inquiry in the United Provinces, viz.: Why do the districts of Bundelkhand suffer less severely from plague than the districts of the Gangetic plain?

*Situation and Boundaries of the Bundelkhand.*

The tract of country in the United Provinces which includes the districts of Jalaun, Jhansi, Hamirpur and Banda which lie to the south of the Jumna is called the Bundelkhand, see Map 4.

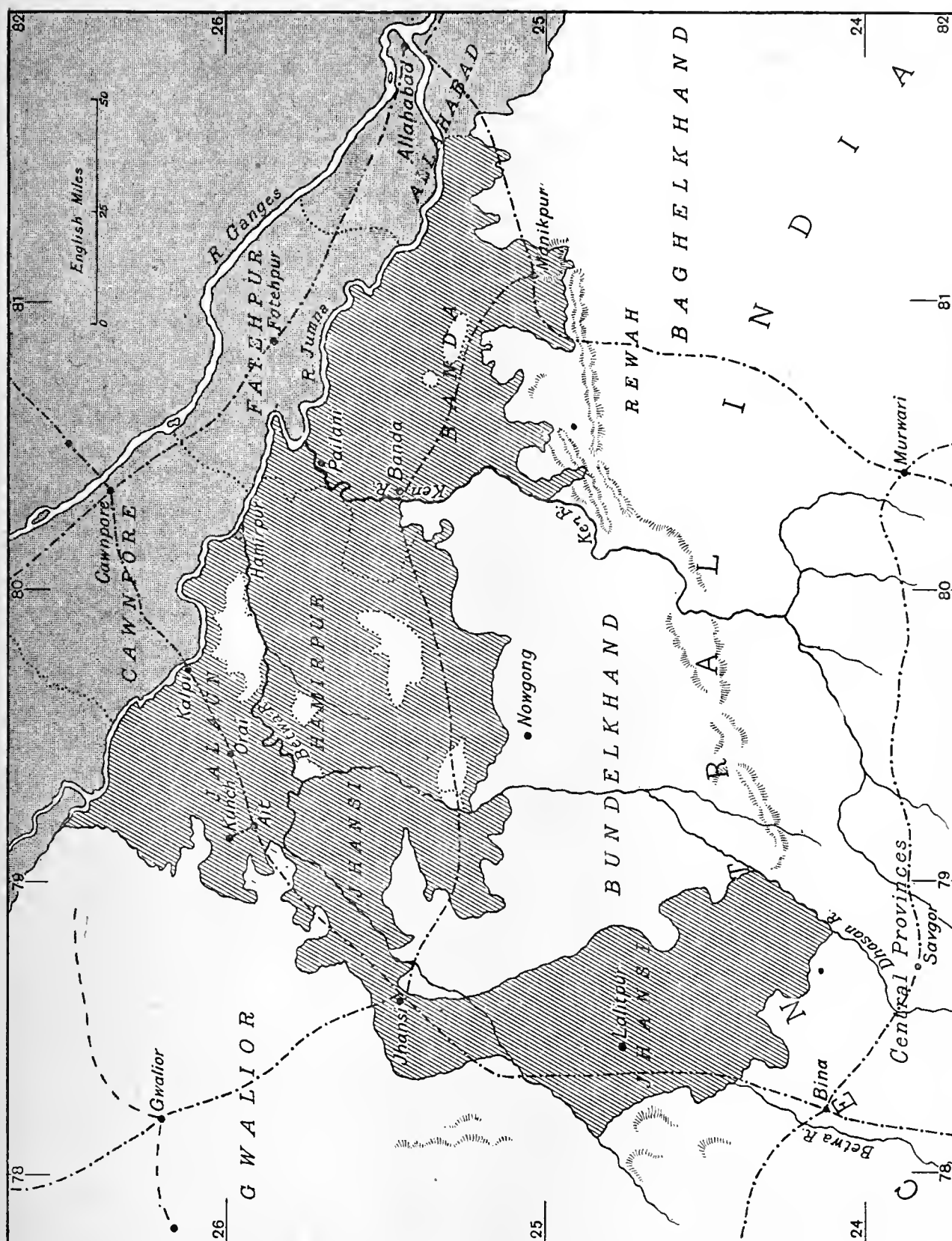
*Physical Geography of the Bundelkhand.*

We have already noted that this tract of country forms a part of the Central India Plateau. The land presents a gradual fall from south-west to north-east but it is broken up, especially in the south, by low-lying rocky spurs of the Vindhya mountains which are covered with stunted trees and jungle. The soil is largely rocky and infertile with considerable patches of the richer type known as black soil which differs entirely from the alluvium of the great plain. The ground water level is very low and canal irrigation hardly exists.

*Climate.*

We have drawn attention to the fact that this tract of country is peculiarly liable to suffer from either an excess or deficiency of rain. Famine therefore often prevails, and for this reason the Bundelkhand ranks as the poorest and most backward portion of the Province.

The only meteorological observing station in that portion of the Bundelkhand which is situated in the United Provinces was at Jhansi till we opened in November 1911 a new observatory in Banda town. Observations have been recorded for many years at Jhansi, and a reference to Table I will show that the climate of Jhansi is drier than that of any other recording station in the Provinces. Except in the months of July, August and September, the mean temperature is higher in Jhansi than in any other observing station. Our records in Banda, which are given in Table XXIX, show that during the period



Map 4. The Bundelkhand Districts of the United Provinces.

of our observations the monthly mean temperature in Banda was even higher than that of Jhansi in the months of April, May, June, July, August and September.

Again a reference to Table I will show that the relative humidity of the atmosphere at Jhansi is lower throughout the year than at any other observing station in the Provinces. In Banda our observations in 1911-12, the only ones available, show that in respect to humidity this place more closely resembles Cawnpore than Jhansi.

It is of interest here to compare the climate of the Bundelkhand with that of the Ballia district, figures for which will be found in Table XXII. It will be observed that, in May and June, both Banda and Jhansi are from five to ten degrees hotter than Ballia, but of probably greater importance, so far as the spread of plague is concerned, is the fact that the relative humidity in Banda during the hot weather is about  $\frac{4}{5}$ , and of Jhansi  $\frac{1}{2}$ , that recorded at Ballia.

TABLE XXIX. *Meteorological observations in Banda in 1911-12.*  
*Means of mean between Maximum and Minimum Temperatures.*

Place	1911 Nov.	Dec.	1912 Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
Banda	63.7 <sup>1</sup>	59.9	61.6	68.5	75.3	86.2	95.4	97.6	86.1	83.9	82.8	81.4 <sup>2</sup>
Jhansi	68.7	62.3	64.0	69.7	76.0	83.4	95.3	93.7	83.9	80.9	79.8	76.8
Allahabad	68.6	60.0	61.5	68.4	75.2	84.2	90.2	91.0	83.7	79.1	78.8	76.5
Cawnpore	67.3	59.9	61.5	67.3	74.3	82.5	88.9	89.8	84.1	82.6	80.5	74.8

8 a.m. Percentage Humidity.												
Banda	88 <sup>1</sup>	88	81	76	52	48	45	53	85	90	82	57 <sup>2</sup>
Jhansi	70	66	77	56	25	25	24	38	76	82	73	56
Allahabad	76	79	80	66	38	39	39	51	82	83	73	62
Cawnpore	75	78	85	72	43	43	41	55	82	86	77	68

<sup>1</sup> 15 days' observation only.

<sup>2</sup> Observations for 20 days only.

#### *Area and Population of the Bundelkhand.*

The area of the Bundelkhand is a little over ten thousand square miles and its inhabitants number rather more than two millions, so that the density of the population is about 200 per square mile. In Table XXX the area, population, density per square mile and the number of towns and villages in each of the districts of the Bundelkhand are given and compared with similar figures for the Ballia district. It will be seen that the density of population is nearly four times as great in Ballia as in the Bundelkhand and that the number of towns and villages is very much larger in this district than in any in the Bundelkhand.

The inhabitants of the Bundelkhand are chiefly Bundelas, a class of people who have a history of their own, distinct from that of the inhabitants of the Gangetic plain; they are more intimately associated with the peoples of Central India.

The people find occupation in their own district; they are on the whole indolent in disposition and do not seek employment in the large industrial centres as do the inhabitants of the Ballia district.

*Communications.*

Except in the Jhansi district the means of communication are limited to a single railway line and a few good roads; Jhansi town is, however, an important railway centre. The Great Indian Peninsular Railway enters the south of the district and divides into two branches at Jhansi, one striking north-west to Agra, the other north-east through the Jalaun district to Cawnpore. A branch line from Jhansi passes through the southern portion of the remaining districts of the Bundelkhand.

TABLE XXX. *Area, Population, Density per square mile, etc. of the Districts of the Bundelkhand compared with those of Ballia.*

Place	Area in square miles	Number of towns	Number of villages	Population	Population per square mile
Jhansi	3628	9	1331	616,759	170
Jalaun	1480	6	837	399,726	270
Banda	3060	5	1188	631,058	209
Hamirpur	2289	7	756	458,542	200
Ballia	1245	13	1784	987,768	793

*Agriculture and Trade.*

While in the United Provinces the autumn crops cover an area only 16 % greater than the spring crops, in the Bundelkhand they are nearly double. A small quantity of grain is exported from the district, but, except in famine years, little grain is imported into it. The trade and commerce of the districts is of little importance; the most noticeable feature is the absence of large central markets. In Jhansi most of the grain imported for local consumption comes from Central India, especially from the Native States of Gwalior and Bhopal. No important fairs or pilgrimages are held in the Bundelkhand.



*The History of Plague in the District.*

Very little plague has occurred in the Bundelkhand. There have been in all, for the ten-years' period 1901-11, 6314 deaths from the disease which gives a mean annual death rate per mille of only 0·3, contrasting with 3 for the whole of the Province and 12 for the Ballia district during the same period.

Tables XXXI, XXXII, XXXIII and XXXIV show the number of deaths from plague for each month in each year for the four districts of this tract. A few words of explanation only are necessary. Plague first appeared in the Jhansi district in October 1902, in a small town called Moth in the north of the district on the main road and railway line between Cawnpore and Jhansi. The disease was probably carried from Cawnpore to this place, for that town was then suffering severely from plague. This little epidemic, which caused 322 deaths, came to an end in the month of May. In the following October, plague

TABLE XXXI. *Showing Plague Deaths each month in the Jhansi District from 1901 to 1911.*

Year	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals
1901-1902	—	—	—	—	—	—	—	—	—	—	—	—	0
1902-1903	—	—	—	3	92	116	23	18	30	33	7	—	322
1903-1904	—	—	—	23	150	32	119	197	185	125	6	—	837
1904-1905	—	—	148	252	57	52	75	37	174	116	25	2	938
1905-1906	—	—	—	—	—	—	—	—	—	—	—	—	0
1906-1907	—	—	—	—	—	—	—	—	—	1	1	2	4
1907-1908	—	—	—	—	—	—	—	—	—	—	—	—	0
1908-1909	—	—	—	—	—	—	—	—	—	—	—	—	0
1909-1910	—	—	—	—	—	—	—	—	—	—	—	—	0
1910-1911	—	—	—	—	—	—	—	1	1	4	3	—	9
Totals	—	—	148	278	299	200	217	253	390	279	42	4	2110

TABLE XXXII. *Showing Plague Deaths each month in the Jalaun District from 1901 to 1911.*

Year	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals
1901-1902	—	—	—	—	—	—	—	—	—	—	—	—	0
1902-1903	—	1	5	2	—	—	1	—	—	1	—	—	10
1903-1904	—	—	—	—	—	—	6	120	487	312	45	1	971
1904-1905	—	39	178	184	186	255	281	240	521	398	81	3	2366
1905-1906	—	—	—	—	—	—	—	1	—	1	—	—	2
1906-1907	—	—	—	—	2	—	—	—	—	1	—	—	3
1907-1908	—	—	—	—	—	—	—	—	19	14	—	—	33
1908-1909	—	—	—	—	—	—	—	—	—	—	—	—	0
1909-1910	—	—	—	—	1	—	—	12	8	14	5	11	51
1910-1911	1	4	—	—	—	—	1	2	8	29	15	—	60
Totals	1	44	183	186	189	255	289	375	1043	770	146	15	3496



TABLE XXXIII. *Showing Plague Deaths each month in the Banda District from 1901 to 1911.*

Year	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals
1901-1902	—	—	—	—	—	—	—	—	1	—	—	—	
1902-1903	—	1	—	—	—	—	2	2	14	23	1	—	43
1903-1904	—	—	—	1	1	—	—	—	4	—	—	—	6
1904-1905	—	—	1	—	—	—	25	24	44	45	9	12	160
1905-1906	—	—	2	46	46	19	27	42	18	—	—	—	200
1906-1907	—	—	—	—	—	—	1	1	1	—	—	—	3
1907-1908	—	—	—	—	—	—	—	—	—	—	—	—	0
1908-1909	—	—	—	—	—	—	—	1	—	—	—	—	1
1909-1910	—	—	—	—	—	—	—	1	—	—	—	—	1
1910-1911	—	—	—	—	—	—	1	1	—	—	—	—	2
Totals	—	1	3	47	47	19	56	72	82	68	10	12	417

TABLE XXXIV. *Showing Plague Deaths each month in the Hamirpur District from 1901 to 1911.*

Year	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Totals
1901-1902	—	—	—	—	—	—	—	—	—	—	—	—	0
1902-1903	—	—	2	1	—	—	—	—	—	4	—	—	7
1903-1904	—	—	—	—	—	2	—	—	—	—	—	—	2
1904-1905	—	—	—	—	—	36	19	20	63	54	4	—	196
1905-1906	—	—	—	—	—	—	—	—	—	—	—	—	0
1906-1907	—	—	—	—	—	—	—	1	1	2	2	—	6
1907-1908	—	—	—	—	—	—	6	10	8	—	—	—	24
1908-1909	—	—	—	—	—	—	—	—	—	—	—	—	0
1909-1910	—	—	—	—	—	—	—	—	—	—	—	—	0
1910-1911	—	—	—	—	—	—	—	14	25	17	—	—	56
Totals	—	—	2	1	—	38	25	45	97	77	6	—	291

again broke out in a small town ten miles south of Moth; and Jhansi city became infected in February. Here the epidemic seems to have been severe and 186 deaths were reported between February and May, but this figure inadequately represents the actual number of deaths from plague, for the mortality from all causes reported in the town during the month of March showed that 656 deaths had occurred while only 93 of these were attributed to plague. No evidence of any other epidemic disease prevailing at the time was obtained, and we ascertained that the normal monthly mortality of Jhansi does not exceed 200.

In September 1904 the southern portion of the district became infected, and the disease was prevalent especially in the town of Lalitpur. In the month of March the disease was fairly widespread over the district, but by May the epidemic was at an end.

From May 1905 till November 1911, plague was practically absent from the district; in the latter month, however, Jhansi city was once

more infected. The epidemic was a very severe one. Deaths from plague were reported as under:

Month	City	Cantonment
November	19	—
December	57	—
January	349	1
February	812	23
March	297	30
April	12	—

Although by February nearly three-quarters of the inhabitants had left the city for the surrounding towns and villages very few of these became infected.

The striking features of the history of plague in the Jhansi district are the three epidemics in the years 1902-3, 1903-4 and 1904-5 and the epidemics in Jhansi town in 1911-12. In seeking for an explanation to account for the irregular distribution of the disease during the years under review two facts of importance have been noted: (1) the unusual atmospheric humidity of the plague years, (2) the coincident epidemics of plague in other parts of Central India, notably in Bhopal, from which State such grain supplies as are required for local consumption are obtained.

Chart I (p. 861) shows: (1) The mean monthly 8 a.m. humidity in the three years 1902-3, 1903-4 and 1904-5, the years in which the plague was epidemic, thin black line. (2) Similar figures for the years in which no plague occurred, 1905-11 (thick black line). (3) The humidity of the year 1911-12, the year of the severe epidemic in Jhansi city.

A reference should also be made to Table XXXV which shows the monthly rainfall in inches at each of the observatories of the United Provinces for 1901-10. There it will be seen that the plague years 1902-3, 1903-4 and 1904-5 were the only years in which rain fell in Jhansi in every month of the year except November and December, and in 1904-5 even in these months some rain fell. Similar phenomena were observed in severe plague years in the Ballia district (page 837), and further evidence in this connection will be adduced for other places in the Province.

It should be noted however that in the year 1906-7 the percentage of humidity in Jhansi was largely in excess of the normal, moreover the epidemic of plague in this year was very severe in the United Provinces as a whole. Throughout that year only four deaths of plague were reported from Jhansi and we are therefore tempted to argue that for some reason or other the remainder of the Province is not a great

source of danger to the Jhansi district from the point of view of plague infection. Additional force is given to this argument when we remember that the people of the Bundelkhand are more intimately connected with the people of Central India than with the United Provinces and that such little grain as is imported into Jhansi comes from Bhopal. It is interesting therefore to note that plague was present in the Bhopal State in 1902-3, 1903-4 and 1904-5, the years in which Jhansi was infected; moreover, Bhopal remained free from plague from the end of 1904 to December 1910. Plague among the rats in Jhansi city was first noted in April 1911. A severe epidemic began in July 1911 in Bhopal and in November of the same year in Jhansi.

TABLE XXXV. *Monthly Rainfall at each of the Observatories of the United Provinces for the years 1901 to 1910 (in inches).*

1901—2												
Observing stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Gorakhpur	16.19	10.37	12.78	—	0.08	0.01	0.05	—	0.18	0.16	0.79	1.42
Benares	4.72	15.1	6.21	—	—	—	0.47	0.07	—	—	0.63	0.58
Allahabad	6.59	11.97	10.45	—	—	—	0.39	0.11	—	—	0.64	1.17
Cawnpore	9.10	11.26	5.73	—	—	—	0.07	0.08	—	—	0.25	0.59
Lucknow	88.78	9.74	12.51	0.31	—	0.06	—	—	—	—	0.79	1.17
Bahraich	8.45	13.17	11.37	—	—	0.21	—	0.03	—	0.21	1.55	0.64
Jhansi	11.50	26.79	1.02	—	—	0.07	1.65	0.19	—	0.01	—	1.72
Agra	3.13	9.22	0.46	0.20	—	0.20	0.02	—	—	0.04	0.05	1.98
Mainpuri	3.48	10.88	1.07	—	—	0.80	—	—	—	0.08	—	0.36
Bareilly	8.71	17.81	6.38	0.18	—	0.57	—	0.09	0.05	0.14	0.26	2.53
Meerut	9.94	11.86	1.83	—	—	0.14	—	—	0.04	0.31	0.56	5.75
Rurki	14.23	15.86	0.33	0.74	—	0.41	—	—	0.14	1.81	1.40	7.41
Dehra Dun	16.50	52.30	6.20	0.72	—	0.41	—	0.33	0.41	1.62	0.99	3.70

1902—3												
Gorakhpur	23.68	5.35	9.28	3.83	—	—	0.26	—	0.02	—	0.72	6.30
Benares	14.49	4.30	7.63	0.01	0.13	—	0.28	—	0.03	0.09	0.17	1.50
Allahabad	18.72	9.27	9.23	—	0.06	—	—	0.06	—	0.06	0.04	0.91
Cawnpore	6.11	3.48	15.97	—	—	—	0.12	—	—	—	—	2.07
Lucknow	14.17	6.53	9.76	0.03	—	—	0.58	0.01	0.03	—	0.03	1.18
Bahraich	14.62	11.01	15.67	0.60	—	—	0.75	—	0.03	—	3.17	7.55
Jhansi	20.33	3.52	6.94	0.24	—	—	0.09	0.05	0.01	0.01	0.30	1.20
Agra	10.86	5.25	4.39	0.04	—	—	0.23	0.10	0.43	—	0.08	0.11
Mainpuri	8.62	6.97	2.67	—	—	—	0.36	—	0.22	—	1.02	1.74
Bareilly	15.06	11.18	9.89	1.17	—	—	1.54	—	0.04	—	0.10	1.32
Meerut	11.17	11.97	2.96	1.17	—	—	1.61	0.02	0.14	—	1.03	—
Rurki	11.61	16.35	4.47	0.08	—	—	1.63	0.06	0.30	0.02	0.05	0.69
Dehra Dun	18.85	21.33	9.85	1.31	—	—	1.66	0.11	0.47	—	0.39	0.98

858 *Epidemiological Observations in United Provinces*

## 1903—4

Observing stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Gorakhpur	10·10	21·07	13·38	15·79	—	—	0·22	—	—	0·02	5·29	9·83
Benares	2·79	10·46	20·19	14·04	—	—	0·82	—	0·29	0·01	0·13	3·38
Allahabad	4·14	18·03	6·12	17·73	—	—	0·30	—	0·31	0·02	0·06	1·00
Cawnpore	2·08	8·45	7·18	11·45	—	—	0·15	0·05	0·18	0·05	0·45	3·04
Lucknow	9·24	16·14	7·42	9·98	—	—	0·20	—	0·24	0·06	0·52	8·00
Bahraich	5·89	22·79	10·57	11·73	—	—	0·69	—	0·03	—	1·60	14·95
Jhansi	6·16	10·30	7·79	8·83	—	—	0·57	0·03	1·67	0·03	0·78	2·59
Agra	4·07	10·27	1·32	7·55	—	—	0·36	—	0·39	—	0·31	3·03
Mainpuri	2·86	8·90	3·50	15·68	—	—	0·05	0·54	0·25	—	0·25	0·89
Bareilly	6·02	10·62	12·94	10·87	—	—	0·25	0·01	0·47	0·11	1·27	3·77
Meerut	7·78	7·20	8·16	0·61	—	—	0·26	—	1·81	0·08	1·98	1·49
Rurki	14·21	13·58	7·10	0·02	—	0·17	0·82	—	3·71	—	1·64	3·97
Dehra Dun	16·20	25·24	14·06	0·79	—	0·98	1·55	0·06	3·44	0·20	2·03	3·73

## 1904—5

Gorakhpur	18·06	16·83	1·65	3·80	0·45	0·75	0·54	1·40	0·61	0·29	1·27	1·03
Benares	15·56	9·49	3·65	4·43	0·49	0·71	0·73	0·35	0·75	0·26	1·12	0·54
Allahabad	12·30	17·61	2·56	5·64	0·55	0·95	0·67	0·20	0·57	0·18	—	0·59
Cawnpore	25·43	15·34	12·18	—	0·91	0·91	0·53	1·46	0·16	0·05	0·29	—
Lucknow	14·23	10·43	6·76	0·66	0·73	0·76	0·31	1·51	0·78	0·16	0·16	0·18
Bahraich	10·52	12·90	4·29	0·76	0·70	1·02	0·64	2·33	1·17	0·31	1·04	1·64
Jhansi	22·31	14·00	1·03	0·02	0·07	1·02	0·53	0·29	0·24	0·02	0·23	0·75
Agra	12·54	15·25	0·84	—	0·37	1·40	0·90	0·97	0·51	—	0·03	0·90
Mainpuri	8·61	12·55	0·98	—	0·31	2·36	0·65	1·28	0·33	0·17	0·02	0·41
Bareilly	11·20	8·37	3·12	—	3·38	1·36	1·77	1·66	0·69	0·25	0·57	0·62
Meerut	7·18	10·77	7·75	0·03	0·49	0·67	2·07	1·19	0·23	0·07	0·18	2·39
Rurki	12·48	6·03	5·29	0·13	0·89	2·24	2·80	2·06	1·20	0·01	0·37	2·02
Dehra Dun	35·07	2·28	6·03	0·55	0·92	2·02	3·37	3·82	1·94	0·20	2·29	5·24

## 1905—6

Gorakhpur	13·22	27·06	6·77	1·65	—	—	0·40	2·22	—	—	0·88	3·90
Benares	12·59	16·63	14·05	0·06	—	—	0·35	1·40	0·63	0·19	—	6·28
Allahabad	11·82	10·01	4·61	—	—	—	0·24	0·49	0·05	—	0·45	2·45
Cawnpore	8·15	7·70	4·33	—	—	0·03	—	1·63	1·03	—	0·07	6·56
Lucknow	10·58	12·99	3·37	—	—	0·03	0·02	2·94	0·18	—	2·38	5·37
Bahraich	9·86	20·47	4·47	—	—	0·35	0·30	4·39	0·08	—	1·13	6·69
Jhansi	6·18	3·28	5·59	—	—	—	—	0·53	0·74	—	0·18	5·75
Agra	4·00	0·51	3·04	—	—	0·03	0·02	1·06	0·97	—	0·04	5·74
Mainpuri	2·10	1·56	6·20	—	—	—	0·11	1·53	1·03	—	—	4·52
Bareilly	8·14	5·36	4·80	—	—	0·12	0·43	3·92	0·99	—	0·22	9·07
Meerut	6·50	2·85	2·38	—	—	0·14	0·13	3·74	0·81	—	0·11	8·86
Rurki	3·60	5·19	3·57	—	0·04	0·52	0·14	6·42	0·84	0·08	0·31	17·20
Dehra Dun	11·77	15·66	9·64	—	0·05	1·00	0·27	6·03	0·60	0·03	0·08	9·80

1906—7

Observing stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Gorakhpur	16.22	18.76	2.15	0.54	—	—	0.20	1.21	1.92	0.32	0.49	2.69
Benares	14.82	10.79	6.31	—	—	—	0.17	6.69	0.66	1.17	0.07	3.80
Allahabad	10.06	8.59	5.44	—	—	—	0.01	3.38	0.20	0.76	0.06	0.70
Cawnpore	6.97	10.50	6.92	0.03	—	—	0.20	2.43	0.31	0.70	0.05	0.88
Lucknow	20.68	10.05	8.02	—	—	—	—	2.06	0.61	0.65	—	0.64
Bahraich	13.75	14.51	2.51	0.20	—	—	0.01	1.80	0.99	0.41	1.06	3.23
Jhansi	8.35	4.72	20.77	0.01	—	—	0.45	3.29	—	0.35	—	1.25
Agra	4.55	3.86	7.76	—	—	0.18	0.32	2.52	0.33	1.32	0.57	0.62
Mainpuri	4.92	5.21	7.87	—	—	0.04	0.22	1.86	—	0.58	0.24	0.22
Bareilly	15.79	8.12	4.54	—	—	0.20	0.53	2.04	0.52	1.57	0.28	0.90
Meerut	10.83	4.58	13.35	—	—	0.75	0.80	2.29	0.71	2.51	0.26	0.05
Rurki	11.39	12.35	8.58	—	—	0.68	1.21	3.68	1.76	1.33	0.65	0.14
Dehra Dun	24.10	27.40	11.71	0.38	—	0.90	2.61	5.05	2.83	0.97	1.08	1.13

1907—8

Gorakhpur	7.14	8.75	2.86	—	—	—	0.28	0.42	0.97	—	—	7.43
Benares	7.85	16.26	1.35	—	—	—	0.50	1.56	0.23	—	0.08	0.41
Allahabad	7.29	14.24	0.02	—	—	—	0.48	0.47	—	—	—	0.20
Cawnpore	8.17	12.73	0.33	—	—	—	0.60	0.26	0.06	—	0.53	0.23
Lucknow	3.52	9.17	0.04	—	—	—	1.12	0.20	0.10	—	0.11	0.47
Bahraich	4.87	4.88	1.22	—	—	—	1.40	0.32	0.20	—	1.07	10.24
Jhansi	5.41	13.96	—	—	—	—	1.32	0.09	0.24	0.05	0.31	1.54
Agra	2.52	15.99	—	—	—	—	1.26	—	—	0.09	0.47	1.26
Mainpuri	4.69	7.28	—	—	—	—	0.79	0.01	—	0.03	0.54	0.48
Bareilly	10.76	10.03	—	—	—	—	0.90	0.13	—	0.09	1.22	10.90
Meerut	1.62	3.57	—	—	—	—	0.83	0.84	—	0.09	0.55	1.89
Rurki	10.66	5.99	—	0.01	—	—	1.91	2.94	—	0.27	0.39	0.72
Dehra Dun	10.82	19.73	1.03	—	—	—	1.17	5.85	0.10	0.75	1.00	6.60

1908—9

Gorakhpur	5.14	7.87	5.88	1.12	—	—	0.20	0.28	—	4.25	0.71	14.67
Benares	9.34	12.45	2.54	1.19	—	0.19	0.51	0.60	—	1.53	0.14	11.83
Allahabad	13.14	16.81	2.38	0.40	—	0.04	0.18	0.24	—	1.29	0.06	10.96
Cawnpore	10.51	17.84	3.90	0.02	—	—	0.20	0.06	—	3.71	—	5.82
Lucknow	16.89	14.06	3.38	0.10	—	—	0.23	0.14	—	7.39	—	4.09
Bahraich	4.53	3.32	4.23	0.87	—	—	0.79	0.20	—	2.93	0.31	9.58
Jhansi	20.91	19.00	3.30	—	—	0.05	0.39	0.14	—	1.45	0.03	5.60
Agra	17.15	13.79	0.69	0.42	—	0.02	1.05	0.21	—	2.81	1.72	2.17
Mainpuri	8.98	12.95	1.37	—	—	—	0.39	0.16	—	1.79	—	5.66
Bareilly	10.76	14.41	1.57	—	—	0.20	0.44	0.38	—	2.45	0.09	9.05
Meerut	14.27	16.17	1.73	—	0.34	0.20	0.71	1.48	—	2.12	0.22	7.96
Rurki	16.68	14.89	0.51	—	0.09	0.09	1.30	0.46	0.02	2.64	—	11.34
Dehra Dun	27.92	26.86	5.11	—	—	0.20	1.38	1.84	0.01	3.85	0.22	1.32

# 860 *Epidemiological Observations in United Provinces*

1909—10

Observing stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Gorakhpur	10·37	13·73	8·45	1·10	—	0·49	0·30	—	—	0·03	2·63	11·59
Benares	16·86	3·85	7·02	0·03	—	0·38	0·31	0·06	0·04	0·02	0·03	3·51
Allahabad	21·26	4·55	7·00	—	—	0·94	0·41	0·05	—	0·01	0·64	3·13
Cawnpore	18·59	4·30	5·19	—	—	1·20	0·12	—	—	—	0·61	2·24
Lucknow	23·47	5·63	5·04	—	—	1·16	0·03	—	—	0·03	2·03	3·22
Bahraich	9·00	17·25	6·24	0·21	—	0·99	0·03	0·02	0·01	—	1·91	7·73
Jhansi	13·38	4·05	4·97	—	—	1·11	—	—	—	0·02	0·24	5·28
Agra	12·71	5·92	2·96	—	—	1·22	0·52	0·16	—	0·15	0·01	1·75
Mainpuri	12·73	6·19	2·34	—	—	0·66	0·36	0·03	—	—	0·70	2·45
Barcilly	17·90	5·16	6·70	0·37	—	1·58	0·36	—	—	0·07	1·86	5·00
Meerut	15·82	11·22	7·07	0·23	—	2·26	0·40	0·26	0·01	0·19	1·26	0·68
Rurki	12·24	7·43	3·86	0·16	—	1·60	1·20	0·17	—	0·14	2·32	4·37
Dehra Dun	42·82	42·71	6·03	0·02	—	1·98	1·72	0·98	—	0·74	1·16	5·13

We were enabled to make a few observations on the rats and fleas of Jhansi towards the close of the epidemic. These are summarised in Table XXXVI. Our observations on the rats were brought to a close by the difficulty in catching a sufficient number of them, the severity of the epidemic being doubtless responsible for this. The large number of fleas caught on the rats is worthy of note. That the epidemic did not spread to neighbouring villages and that it rapidly terminated in March and April was probably due to the very dry weather and low percentage humidity in those months, see Chart I.

TABLE XXXVI. *Observations on the Rats and Fleas of Jhansi.*

Week ending	No. of <i>Mus rattus</i> examined for fleas	No. of fleas	Av. fleas per <i>Mus rattus</i>	<i>Mus rattus</i> caught per 100 traps set	Plague infected
17 Feb. 1912	110	1399	12·7	17	5 (4 dead)
24 „	130	1694	13	12	5 <i>Mus rattus</i> 8 squirrels
2 March 1912	48	866	18	4	1 <i>Mus rattus</i> 3 squirrels
16 „	10	77	7·7	1	1 squirrel

(All *X. cheopis*)

The salient features of the plague epidemics in Jhansi may be summed up as follows:

(1) Epidemics have only occurred when the atmospheric humidity has been considerably above the normal percentage.

(2) Epidemics in Jhansi have coincided with epidemics in the Native States of Bhopal and Gwalior, such small imports of grain as are brought to Jhansi for local consumption being derived from these States.

(3) It is noteworthy that in 1906-7, although the atmospheric humidity was high, and plague was severe and widespread in other

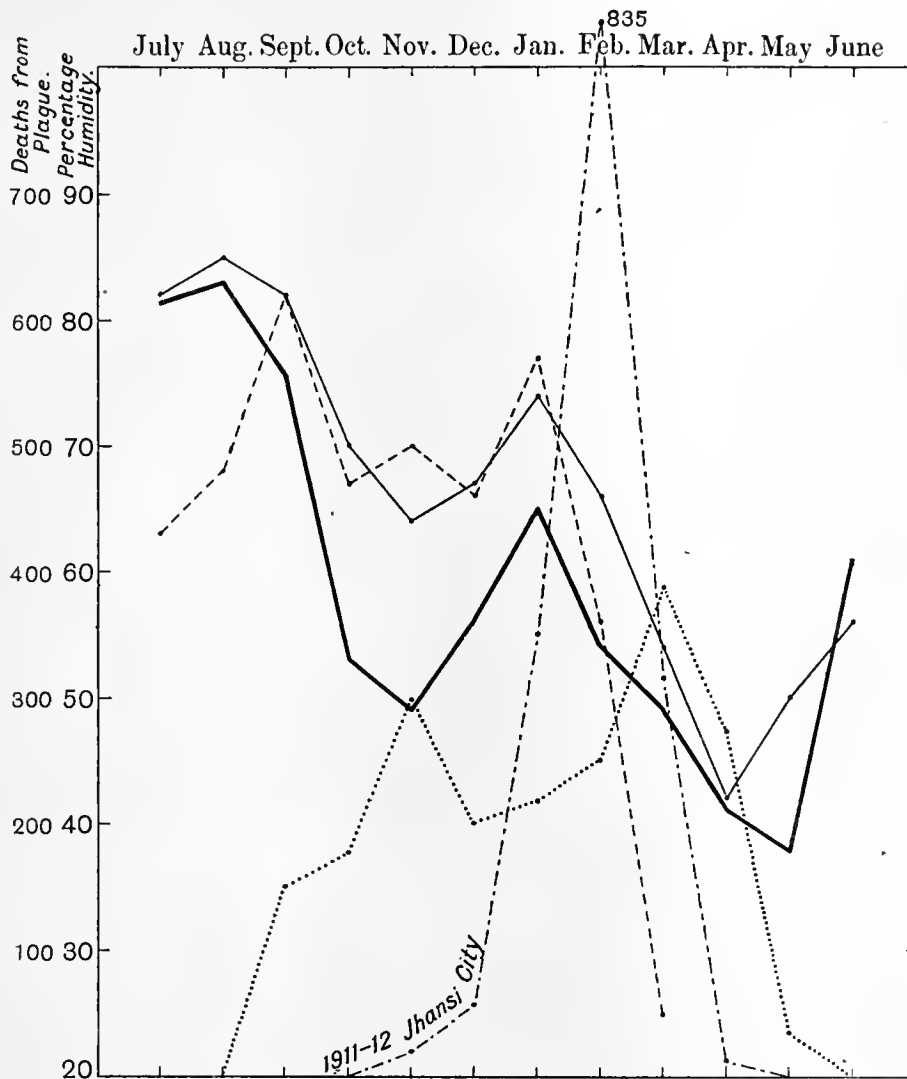


Chart I. Jhansi.

- Mean monthly 8 a.m. percentage humidity: plague years, 1902-3, 1903-4 and 1904-5.
- Percentage humidity of six non-plague years, 1905 to 1911.
- Percentage humidity 1911-12; severe outbreak in Jhansi City.
- ..... Monthly incidence of human plague. Deaths in the three small epidemics—1902-3, 1903-4 and 1904-5 (Jhansi District).
- . - . - . Outbreak in Jhansi City, 1911-12.

parts of the United Provinces, the Jhansi district escaped infection together with the Central Indian Native States.

(4) The epidemic in Jhansi city in 1911-12 failed to spread throughout the district although nearly three-quarters of the inhabitants had

taken refuge in the surrounding villages; it is possible that this was due to the very dry weather which prevailed in March and apparently rapidly brought the epidemic to a close.

The history of plague in the remaining districts of the Bundelkhand corresponds very closely to that of the Jhansi district. In none of the districts has the disease been at all widespread, such epidemics as have occurred were for the most part confined to the years 1903-4 and 1904-5, years of exceptional humidity, see Tables XXXI to XXXIV, also Tables XXXV and XXXVIII.

In the Banda district however the epidemic which was associated with the largest number of deaths occurred in the year 1905-6, Table XXXIII. This epidemic, like the other in 1904-5 in this district, was confined for the most part to the neighbourhood of Rajapura, a trading centre of some importance. It is noteworthy that the year 1905 was one of local famine and it is stated that grain was imported into this district to an unusual extent.

#### *Observations on Rats and Fleas in Banda.*

In September 1911 we decided to start a laboratory in Banda town with the object of obtaining some information regarding the rats and fleas in the Bundelkhand. The work in this connection was carried out on the same lines as that done in other places in the United Provinces and the results have been tabulated in Table XXXVII, which can be compared with Tables XVII and XIX which refer to Lucknow and Cawnpore respectively.

A reference to these tables will show:

(1) that *Mus rattus* is the common house rat of Banda and that judging from the number caught per 100 traps set, they were almost as abundant as they were in Cawnpore and rather more abundant than they were in Lucknow.

(2) that the average number of fleas per rat was remarkably high throughout the whole of the cold season; fleas in fact at this time were more numerous upon the rats in Banda than in any other place investigated in the United Provinces. During the months of May, June and July, however, fleas were less numerous on the rats here than in Ballia, the figures being approximately:

Place	May	June	July
Banda	6.4	3.1	4.3
Ballia	6.7	6.2	5.6



TABLE XXXVII. Observations on Rats and Fleas in Banda.

	Week ending	Total no. of rats examined	No. of <i>Mus rattus</i> on which fleas were counted	Total no. of fleas	Average no. of fleas per <i>Mus rattus</i>	Total no. of <i>Ceratophyllus fasciatus</i>	No. of adult female <i>Mus rattus</i>	No. of pregnant <i>Mus rattus</i>	Percentage of adult females pregnant	Young <i>Mus rattus</i> (70 gms. and under)	Percentage young of total	No. of <i>Mus rattus</i> per 100 traps set
Sept.	23, 1911	272	255	3544	13.9	—	—	—	—	121	40	75
"	30, "	295	259	3637	14.0	—	94	—	—	92	31	54
Oct.	7, "	312	304	5040	16.6	—	132	—	—	61	20	67.9
"	14, "	399	391	6672	17.0	—	161	—	—	112	28	71.6
"	21, "	342	333	7334	22.0	—	133	26	20	130	38.6	67
"	28, "	393	370	7256	19	—	107	13	12.1	136	36.2	66.9
Nov.	4, "	366	329	6270	19.2	—	133	12	9.0	107	30.6	60.3
"	11, "	636	536	8985	16.7	—	241	15	6.2	169	27.0	114.7
"	18, "	477	419	6393	15.3	—	176	11	6.2	108	23.6	77.9
"	25, "	448	401	4538	11.3	—	149	10	6.6	117	27.6	76.8
Dec.	2, "	286	273	3487	12.8	—	106	6	5.7	75	27.4	53.9
"	9, "	284	267	3695	13.8	—	127	11	8.7	31	11.5	45.8
"	16, "	213	121	1626	13.4	—	78	9	11.5	47	25	38.7
"	23, "	229	214	2842	13.3	—	98	19	19.4	35	16.3	36.9
"	30, "	215	200	2793	13.9	—	78	11	14.1	27	13.5	43.6
Jan.	6, 1912	259	243	3750	15.4	—	105	7	6.7	38	15.6	50.7
"	13, "	266	256	2702	10.6	—	111	15	13.5	31	12.1	45.1
"	20, "	287	272	3837	14.1	—	115	17	14.8	38	13.9	46.7
"	27, "	202	184	2297	12.5	—	80	15	18.7	18	9.8	31.8
Feb.	3, "	247	236	4093	17.3	—	120	22	18.3	23	9.7	40.3
"	10, "	246	233	3147	13.6	—	90	23	25.6	54	23.1	39.8
"	17, "	315	303	4798	15.8	—	141	39	27.6	32	10.6	51.5
"	24, "	233	220	3563	16.2	—	76	21	27.6	38	17.3	37.4
March	2, "	213	202	2755	13.6	—	80	28	35.0	30	14.9	34.8
"	9, "	239	234	3196	13.7	—	84	30	35.7	57	24.4	39.8
"	16, "	266	260	4057	15.7	—	90	32	35.6	83	31.5	44.2
"	23, "	204	197	2587	13.1	—	92	31	33.7	48	24.4	34.2
"	30, "	257	248	3534	14.2	—	74	22	29.7	99	39.9	42.2
April	6, "	192	185	2049	11.1	—	70	29	41.4	63	34.1	31.8
"	13, "	186	179	1989	11.1	—	66	22	33.3	61	34.1	30.9
"	20, "	299	292	3224	11.0	—	85	38	44.7	149	50.9	50.2
"	27, "	123	114	1143	10.0	—	18	6	33.3	—	—	31.1
May	4, "	131	119	777	6.5	—	—	—	—	—	—	37.7
"	11, "	179	155	1248	8.05	—	41	13	31.7	101	57.7	33.6
"	18, "	180	142	612	4.3	—	41	18	43.9	94	52.2	30.9
"	25, "	106	91	443	4.8	—	29	16	55.1	53	50.0	20.6
June	1, "	136	136	1047	7.7	—	36	11	30.5	70	51.1	23.7
"	8, "	83	73	201	2.7	—	18	11	61.1	46	56.1	17.6
"	15, "	151	151	609	4.03	—	38	22	57.8	88	58.2	26.3
"	22, "	178	178	987	5.5	—	38	11	28.9	112	62.9	31.7
"	29, "	182	175	433	2.4	—	52	19	36.5	85	46.7	31.6
July	6, "	247	247	1122	4.5	—	54	13	24.07	148	59.9	42.8
"	13, "	101	101	403	4.0	—	23	9	39.1	49	48.5	18.5
"	20, "	191	191	764	4.0	—	71	25	35.2	69	36.1	33.1
"	27, "	183	183	853	4.6	—	78	24	30.7	57	31.1	31.7
Aug.	3, "	99	99	881	8.9	—	45	15	33.3	28	28.2	17.1
"	10, "	134	161	2420	15.03	—	61	21	34.4	63	38.4	33.9
"	17, "	220	220	2742	12.4	—	83	35	42.1	89	40.4	45.5
"	24, "	217	217	3134	14.4	—	65	27	43.07	67	30.8	40.7
"	31, "	124	123	2547	20.7	—	43	19	44.1	47	38.2	24.5
Sept.	7, "	270	270	5211	19.3	—	75	21	28	111	41.1	53.1
"	14, "	233	233	4545	19.5	—	75	29	38.6	99	42.5	43.1

## 864 *Epidemiological Observations in United Provinces*

(3) that all the rat fleas were *Xenopsylla cheopis*; no *Ceratophyllus fasciatus* were found.

(4) that the number of rats caught in the houses was fewer in May and June than at any other time of the year. Breeding was most active, as indicated by the number of pregnant females found, in March

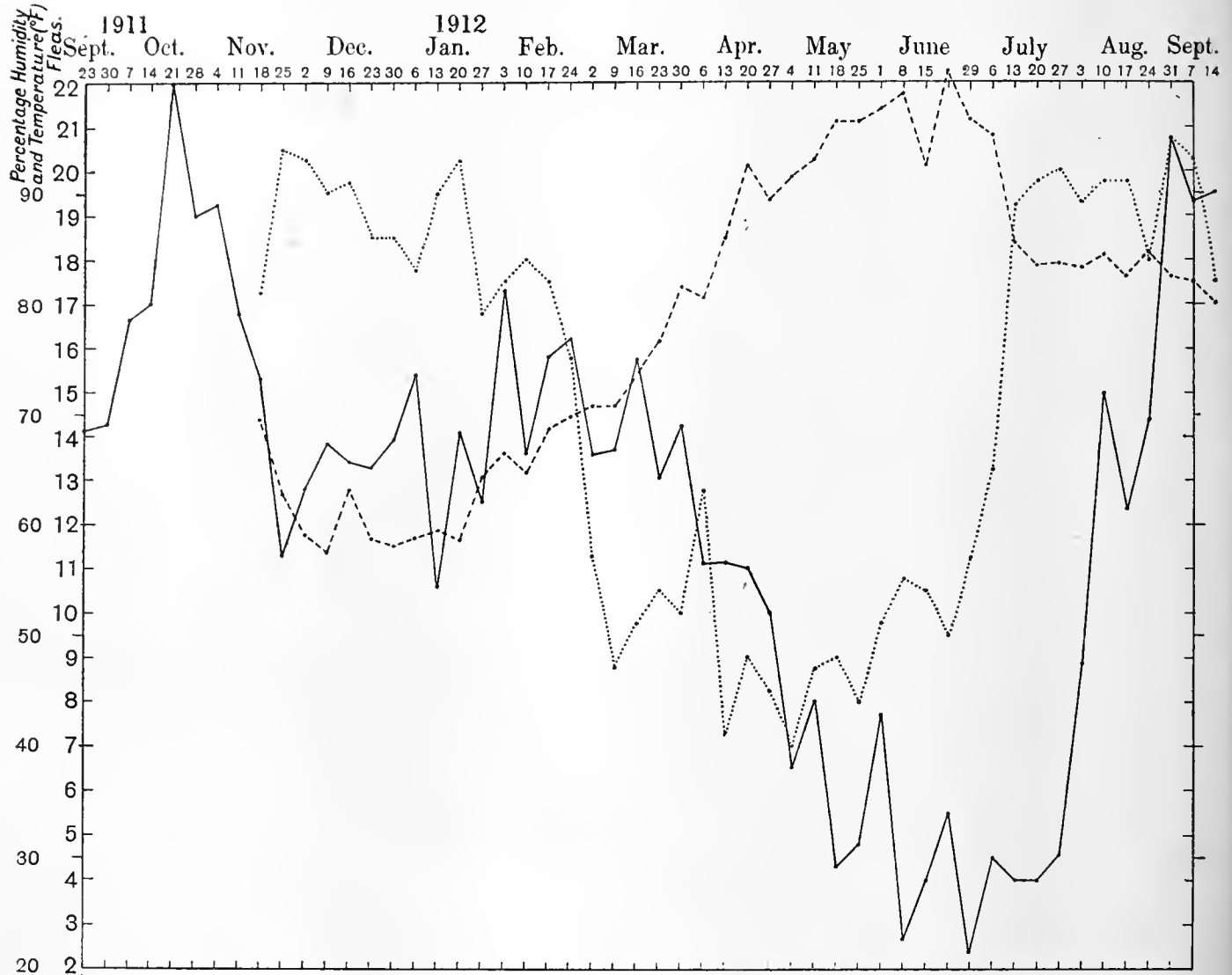


Chart II. Banda.

— Average fleas per *Mus rattus*.  
 ..... Percentage humidity.  
 ----- Mean of mean maximum and minimum temperatures.

and April, while the largest proportion of young to old rats was noted in May and June.

Chart II shows in a graphic manner the relation of temperature and humidity to the number of fleas found on the rats.

*Susceptibility of Banda rats to Plague.*

Two experiments were made to test the susceptibility of the Banda rats to plague. These experiments are detailed on pages 250 and 254 of Plague Supplement II, to the *Journal of Hygiene*, January 1913, and show that Banda rats are almost as susceptible to plague as Madras rats are. In one of the experiments Banda rats were compared with rats from Lucknow and Cawnpore. This experiment showed that while 98 % of the Banda rats died from a dose of 20,000 plague bacilli injected subcutaneously only 32 % of the Lucknow rats and 20 % of the Cawnpore rats died from the same dose. So far then as rats and fleas are concerned, Banda appears to be a place very suitable for the development of a plague epidemic. As a matter of fact plague broke out in Banda town in the week ending 18th October 1913, some time after we had closed the observations which are recorded above. From that date to the week ending the 7th March 1914 when the epidemic closed, only 84 deaths from this disease were reported among a population of 22,565. It should be stated however that in the opinion of the Civil Surgeons many more deaths from plague than those reported occurred, but no accurate figures are available; there is no doubt however that although many persons left the town, the disease was not carried into the surrounding villages, and in this connection it is noteworthy that the year was one of exceptional dryness.

*The consideration of the question of the comparative immunity  
from plague of the Bundelkhand.*

The freedom from plague of a large tract of country does not depend, as a rule, on any single feature which is inimical to the disease, such as the climate, the number of people or their habits, agriculture or trade; nor yet on the number or immunity of its rats to plague, nor on the number of fleas found on them. The freedom from or susceptibility to plague of a district or country depends rather on a combination, in varying degrees, of some or all of the features enumerated above. In attempting therefore to solve the problem at present before us we can but detail the various features which appear to us to have some influence favourable or otherwise to the prevalence of plague. The relative value of each of the features which make a place more or less susceptible to plague can only be surmised or imagined. It may well be, however, that other unexplained factors are at work, without knowledge of which a completely satisfactory explanation of the geographical distribution

of plague cannot be arrived at. With this *caveat* a picture of the influence of all the above features working together can best be drawn if we attempt to compare and contrast each feature as found in:

- (a) the comparatively plague-free Bundelkhand with
- (b) the severely smitten Ballia district.

Taking first the influence of climate on plague prevalence we find that, generally speaking, the Jhansi district of the Bundelkhand is hotter and drier than any other part of the United Provinces, especially in the winter months. At this season the climate though favourable for plague is less suitable for the development of epidemics of the disease than that of the districts in the Gangetic plain. We find that the disease in Jhansi has only become epidemic at this season in years of exceptional humidity. In the hot weather the climate is intensely hot and dry, epidemics at this season, therefore, rapidly decline and terminate so that very few deaths from plague are reported in July and August, the commencement of the favourable season. Contrast with this the climate of Ballia in May and June; here it is cooler and moister than in any other part of the Province situated in the Gangetic plain or Bundelkhand, epidemics therefore decline more slowly and linger through this the usually unfavourable season. Many deaths are therefore reported in July and August, the commencement of the favourable season. Table XXVI shows that while only 46 deaths from plague were recorded in the Bundelkhand during the months of July and August in the ten-year period 1901-10, no fewer than 2228 deaths were noted in the same months during the same period in the comparatively small area included in the Ballia district. The Jhansi district of Bundelkhand therefore appears to start each plague season with a clean slate while scattered foci of infection linger at this time in the Ballia district. With regard to Banda, the other district of the Bundelkhand for which we possess temperature and humidity data during 1911-12, the climate would appear to be not very different from that of Cawnpore, so that the absence of severe epidemic plague cannot be attributed to the influence of climate. It is, however, open to question how far these data accurately represent the normal climatic conditions of the district, but unfortunately they are the only figures available.

With regard to the high density of population of Ballia, as compared with the Bundelkhand, that such high density of population should, other conditions being equal, favour a high mortality from plague appears almost axiomatic on any theory of spread. We find, however, that no close relation exists between density of population and mortality

from plague even in the case of districts in the same province. (See Table XXVIII.)

Then again the Bundelas seldom work in the great industrial centres, which are often hot-beds of plague, although they freely visit friends in Cawnpore and Allahabad, which are not far distant. The people of Ballia, however, are in the habit of working in places outside their own districts; they return to their homes from time to time, especially when sick or scared by epidemic diseases such as plague, so that they are liable to bring infection with them. With regard to this question of personal communication between the people of the Bundelkhand and other infected districts, Mr Silberrand, Collector of Jhansi, after carrying out an exhaustive enquiry on our behalf, concluded that, although less than many other parts of the United Provinces, there was sufficient personal intercourse with infected districts to render Jhansi liable to infection from without.

Again in the Bundelkhand the people live on the grain grown on the land and import little agricultural produce; trade therefore, which often carries infection in its wake, is poor in the Bundelkhand.

Observations made in Jhansi and Banda on rats and fleas showed that rats were present in abundance throughout the districts and that the flea infestation of the Banda rats was higher than in any other district in the United Provinces. The Banda rats, moreover, were found to be very susceptible to plague, when compared with rats from Lucknow or Cawnpore. Nevertheless, the presence of a comparatively large number of very susceptible rats infested with many fleas found in the Bundelkhand is of little moment in favouring plague, for infection seldom reaches them. Severe epidemics may occur, and have occurred, when infection has been successfully imported (*e.g.* the epidemic in Jhansi in 1911), but infection must be implanted early in the season to produce a severe epidemic, for the favourable period for plague is comparatively short. The disease does not tend to spread in the Bundelkhand except in unusually humid years, very dry and hot climate is unfavourable to the prolonged existence of rat fleas when separated from their host.

## CHAPTER VI

THE SEVERE EPIDEMICS OF PLAGUE IN MUTTRA IN 1904-5  
AND IN MUZAFFARNAGAR IN 1906-7

We have lastly to consider the conditions associated with the severe epidemics of plague which occurred in the Muttra district in the year 1904-5 when 51,002 deaths were attributed to plague, giving a death rate of 66.84 per mille, the highest death rate from plague ever recorded in any district in the United Provinces; and in Muzaffarnagar in 1906-7 when 49,745 deaths from the disease were reported, giving a death rate of 56.72 per mille.

*The Muttra District.*

Muttra is one of the districts of the Agra division of the Province; the other districts, which make up this division, are Agra and Etawah, situated to the south of Muttra, and Mainpuri, Etah and Farukhabad to the east of it. To the north of Muttra lies the Aligarh district of the Meerut division of the Province and the Gurgaon district which forms part of the Punjab. An examination of Table VIII shows that in the year 1904-5 the whole of the Agra division as well as the district of Aligarh suffered from a severe epidemic of plague. The epidemic was not confined to these districts of the United Provinces only but extended also into the Punjab district of Gurgaon. The Muttra district however seemed to be the focus or centre of the epidemic.

As a rule the climate of the Muttra district is very dry and hot, great extremes of temperature however occur. In January the mean temperature falls to 60°, while in June it rises to over 93°. The annual rainfall averages about 26 inches, but variations from year to year are large, the fall has been less than 16 inches and has reached nearly 36. The area of the district is 1445 sq. miles. Fourteen towns and 837 villages are situated in it having a population of 763,099, so that there are approximately 528 persons to the square mile. The density of the population is thus higher than the provincial average but not so high as in the Ballia and other districts in the east of the Province.

The inhabitants are good agriculturists and trade and commerce flourish in the district. Muttra city is an important depot for through traffic. Communications are good in the district, for a number of railway lines traverse it and it is well supplied with roads. Save perhaps in respect to the dry climate Muttra is a district suitable for the development of plague, so far as we can judge from the above facts.

The study we have made of plague in the Ballia district and in the districts of the Bundelkhand has shown that the severe epidemics of plague in these districts have been associated with an unusually high degree of atmospheric humidity during the winter months. We observed that severe epidemics occurred when the rainfall of the winter months was above the average. This finding led us to inquire into the climatic conditions of the Muttra district in the year 1904-5. Unfortunately meteorological records are not available for the Muttra district itself, which does not possess an observatory, but the rainfall figures for the Muttra city show that the winter rains in 1904-5 were considerably in excess of the normal. Thus we find the departures from the normal monthly rainfall in inches in Muttra were in November, plus 0.49; in December, plus 0.59; in January, plus 0.94; in February, plus 0.31, or an excess of 2.32 inches over the normal rainfall of 1.21 inches during these months. A considerable increase over the normal rainfall of the winter months was also recorded at the Agra observatory, and here figures showing the monthly percentage humidity of the period under review are also available; these figures as well as those for the other years between 1901 and 1911 are recorded in Table XXXVIII. In Chart III figures showing the mean monthly percentage humidity read at 8 a.m. for the year 1904-5 are compared with the average of similar figures for all other years than 1904-5 between 1903 and 1911. A further contrast is depicted between similar figures for the year 1905-6, the year in which plague was least prevalent in the Agra division, and the figures for the severe plague year 1904-5. This chart shows clearly that the atmospheric humidity in 1904-5 was considerably in excess of the normal and that the difference between the humidity in a severe plague year and in a mild plague year is even more striking. We think we have in the unusual humidity of the winter months of 1904-5 a sufficient explanation for the unusual severity of plague in the Muttra district in this year, especially when this observation is supported by similar ones already adduced in connection with our inquiries in the Ballia district and in the Bundelkhand. Moreover, further evidence of a similar nature will be presently brought forward for other severe

## 870 *Epidemiological Observations in United Provinces*

epidemics in other places in the Province. We need only add that experiments made in connection with the effect of temperature and humidity on the number of hours a flea can live when separated from its host are in consonance with this finding, for in a climate such as

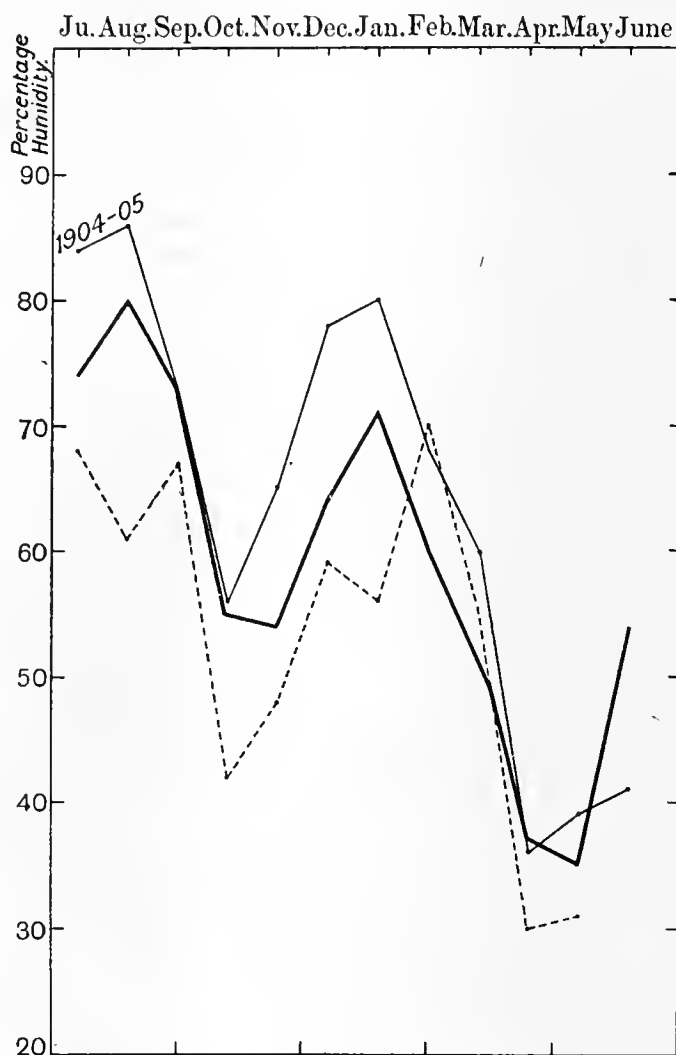


Chart III. Agra Observatory.

————— Percentage humidity: severe plague year in Agra Division, 1904-5.  
 ————— " " " plague years 1903-11.  
 - - - - - " " " year of minimum plague incidence, 1905-6.

that of Muttra in the winter and spring months of 1904-5 rat fleas could live for some days away from their normal hosts and could therefore convey infection more frequently and efficiently both to rats and human beings.



TABLE XXXVIII.

HUMIDITY TABLES. *Mean percentage Humidity at 8 a.m.*

1901—2

Stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Rurki	74	87	80	79	76	82	80	61	48	40	45	58
Meerut	77	87	71	68	71	74	71	55	46	38	43	62
Bareilly	83	89	82	81	78	82	82	63	51	42	54	66
Agra	72	85	67	56	54	62	64	53	45	42	44	56
Mainpuri	70	85	74	60	58	66	72	52	39	34	37	48
Cawnpore	74	87	76	66	62	73	78	57	42	39	50	51
Bahraich	82	86	83	75	76	85	87	69	58	55	58	69
Lucknow	79	89	80	74	76	79	84	62	46	41	55	53
Allahabad	77	87	83	72	67	77	83	62	47	42	49	49
Benares	76	88	85	75	73	75	83	69	55	57	57	55
Gorakhpur	86	89	83	73	73	79	84	73	59	63	70	74
Jhansi	73	88	77	67	62	72	69	61	48	47	47	53
Patna	86	89	81	77	74	74	79	58	57	65	65	73

1902—3

Rurki	83	83	83	78	80	82	85	75	57	38	33	50
Meerut	83	80	78	72	69	65	72	63	52	37	35	50
Bareilly	91	88	89	84	78	80	80	75	57	38	39	67
Agra	79	74	79	63	54	60	64	55	43	31	36	44
Mainpuri	80	77	79	62	58	62	70	66	49	66	33	51
Cawnpore	85	79	86	67	64	69	72	58	42	27	37	62
Bahraich	83	86	86	77	73	76	81	69	54	60	96	89
Lucknow	84	83	84	74	72	69	78	64	48	26	36	65
Allahabad	85	82	85	72	74	77	79	65	40	29	34	59
Benares	85	83	86	77	76	74	81	73	45	33	38	67
Gorakhpur	93	91	91	87	81	83	88	74	53	34	54	78
Jhansi	86	80	83	71	65	59	70	63	38	36	46	53
Patna	88	87	89	79	73	72	81	67	53	43	60	77

1903—4

Rurki	65	86	88	81	76	83	87	73	71	34	45	58
Meerut	66	85	84	75	63	66	78	62	66	36	51	54
Bareilly	78	90	89	88	75	78	84	68	64	42	59	66
Agra	66	84	79	73	56	63	73	58	58	30	42	51
Mainpuri	62	86	87	81	65	69	78	65	60	37	42	54
Cawnpore	71	87	87	83	65	75	81	72	63	43	50	61
Bahraich	84	90	86	84	83	87	94	85	74	64	89	—
Lucknow	69	87	87	86	70	76	79	67	55	30	51	64
Allahabad	66	84	86	86	74	77	83	72	59	34	53	65
Benares	71	85	87	85	74	80	85	74	62	54	59	70
Gorakhpur	82	89	88	87	74	81	81	68	57	55	71	79
Jhansi	74	86	86	80	61	63	74	67	63	44	53	60
Patna	80	89	87	85	74	80	84	71	52	55	70	80

## 872 *Epidemiological Observations in United Provinces*

### 1904—5

Stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Rurki	84	85	78	74	80	89	88	84	71	45	43	55
Meerut	81	85	79	70	74	83	83	76	64	43	43	53
Bareilly	87	89	82	73	81	91	89	88	79	56	60	59
Agra	84	86	73	56	65	78	80	69	60	36	39	41
Mainpuri	84	86	75	61	73	85	84	75	65	46	52	47
Cawnpore	84	88	78	64	70	86	85	68	58	40	47	41
Bahraich	92	89	79	77	81	—	94	85	80	68	68	—
Lucknow	84	87	79	69	76	89	86	70	60	37	50	44
Allahabad	87	90	80	71	76	90	87	76	66	44	53	47
Benares	85	89	80	73	76	87	84	76	66	53	56	48
Gorakhpur	89	90	80	75	78	89	85	77	62	60	66	71
Jhansi	87	90	77	60	66	79	79	69	62	47	50	54
Patna	88	90	80	73	78	85	84	69	64	54	69	73

### 1905—6

Rurki	77	88	83	72	67	79	80	85	72	40	34	54
Meerut	72	75	74	61	64	67	62	75	65	28	38	55
Bareilly	80	91	84	79	73	86	79	87	70	45	49	53
Agra	68	62	67	42	48	59	56	71	55	30	31	54
Mainpuri	74	79	78	67	74	81	71	82	62	34	35	53
Cawnpore	75	81	81	54	57	65	67	74	54	27	39	58
Bahraich	83	95	84	75	73	81	79	83	65	34	50	65
Lucknow	78	89	83	63	59	73	76	72	56	25	43	63
Allahabad	78	88	82	62	59	65	66	76	47	22	40	53
Benares	80	88	85	71	71	74	78	82	60	44	53	65
Gorakhpur	84	92	85	77	73	78	75	77	61	42	56	76
Jhansi	86	76	73	53	55	63	62	69	63	54	58	73
Patna	84	91	88	76	69	72	76	83	64	38	60	74

### 1906—7

Rurki	81	88	87	80	86	93	92	91	75	62	40	43
Meerut	78	83	84	69	71	84	87	84	71	66	47	45
Bareilly	—	—	—	79	78	87	89	90	71	60	44	48
Agra	79	78	81	57	57	72	76	77	57	48	33	45
Mainpuri	81	85	86	69	66	81	82	81	67	51	35	45
Cawnpore	85	83	81	66	68	86	85	87	66	60	38	48
Bahraich	87	88	79	73	75	87	87	83	69	56	49	61
Lucknow	88	87	82	69	69	81	82	79	62	47	39	56
Allahabad	81	81	80	70	66	80	78	78	54	45	32	48
Benares	84	83	82	73	68	80	84	81	61	61	49	66
Gorakhpur	89	88	79	75	88	81	83	77	61	60	52	74
Jhansi	84	83	86	71	59	70	73	78	69	63	56	66
Patna	86	87	84	75	69	77	79	79	63	56	54	75

1907—8

Stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Rurki	67	83	67	59	69	72	85	81	51	40	33	46
Meerut	64	80	56	53	58	70	77	65	43	41	45	51
Bareilly	72	85	64	60	66	74	83	71	65	50	50	52
Agra	61	84	55	44	47	52	72	50	39	36	40	51
Mainpuri	64	88	61	48	59	65	76	59	42	31	42	56
Cawnpore	64	87	60	47	53	70	81	57	41	31	37	48
Bahraich	79	80	68	67	61	66	86	75	61	43	54	71
Lucknow	73	89	63	51	55	71	79	60	36	31	41	55
Allahabad	65	84	64	47	56	68	76	55	36	23	33	44
Benares	73	88	72	51	59	73	81	68	59	37	48	54
Gorakhpur	85	87	76	68	65	66	76	62	51	52	48	71
Jhansi	78	91	73	—	36	42	65	41	38	29	24	39
Patna	87	85	81	63	61	64	77	68	48	42	58	74

1908—9

Rurki	84	85	83	72	75	81	84	74	52	54	35	75
Meerut	87	88	75	63	72	80	76	65	52	56	45	74
Bareilly	84	86	77	74	66	72	72	61	54	66	59	90
Agra	89	91	74	55	60	66	72	53	38	54	37	66
Mainpuri	81	86	74	61	60	69	71	62	47	59	51	85
Cawnpore	83	88	76	53	60	73	70	58	34	52	38	74
Bahraich	80	79	78	67	81	85	82	65	41	54	43	75
Lucknow	84	87	79	60	66	77	67	58	33	60	44	76
Allahabad	80	85	75	54	58	71	65	58	28	48	33	76
Benares	81	88	80	59	61	80	75	68	38	64	42	80
Gorakhpur	92	80	78	66	67	81	73	63	37	62	57	85
Jhansi	85	89	67	45	47	47	59	50	46	52	39	66
Patna	82	83	82	62	62	71	68	64	32	60	58	83

1909—10

Rurki	87	85	82	74	78	91	92	77	57	41	40	65
Meerut	86	83	83	69	76	89	89	74	51	50	57	60
Bareilly	92	84	80	66	73	87	85	74	61	57	59	77
Agra	86	78	75	49	53	75	71	54	39	31	33	59
Mainpuri	94	87	83	56	65	82	82	61	43	36	37	67
Cawnpore	89	82	77	55	64	80	83	56	36	29	39	68
Bahraich	84	86	81	72	77	85	94	70	52	39	51	77
Lucknow	89	85	83	62	68	78	78	54	40	39	43	73
Allahabad	86	79	80	58	64	81	79	53	31	28	36	63
Benares	88	83	82	63	66	82	81	57	40	29	50	74
Gorakhpur	87	89	86	80	81	86	87	69	59	74	79	86
Jhansi	85	79	78	46	49	68	58	41	29	27	25	63
Patna	86	86	82	72	66	76	76	61	50	47	65	78

1910—11

Stations	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Rurki	84.	87	87	81	84	80	92	82	75	49	42	71
Meerut	74	84	83	78	70	69	88	71	68	37	32	56
Bareilly	83	89	86	84	78	73	87	76	73	50	60	79
Agra	69	80	80	63	58	59	78	54	56	32	28	53
Mainpuri	70	84	88	77	69	70	84	68	66	41	36	53
Cawnpore	76	86	84	67	66	69	83	62	59	31	36	53
Bahraich	86	87	87	85	83	86	90	81	76	49	56	68
Lucknow	79	85	87	72	67	69	83	61	60	36	54	61
Allahabad	72	84	85	69	69	72	79	57	58	37	43	60
Benares	75	87	86	76	77	77	85	66	65	42	49	71
Gorakhpur	88	89	86	78	79	81	87	73	64	46	61	74
Jhansi	71	81	76	52	48	45	75	44	48	47	23	57
Patna	84	86	85	76	72	73	—	—	—	—	—	—

*The Muzaffarnagar District.*

The Muzaffarnagar district was the centre of a severe epidemic of plague in 1906-7 which also extended into the adjacent districts of Saharanpur, Bijnor, Moradabad and Meerut as well as into the Punjab districts of Umballa, Karnal and Delhi.

The Muzaffarnagar district has however suffered from other severe epidemics of plague, especially in the years 1910-11 and to a less extent in 1904-5. Over the whole period of ten years 1901-1911 the death rate from plague per mille per annum has averaged 11.20 so that the mortality in this district follows closely on that of the Ballia district, which has an average death rate from plague per mille per annum for the same period of 12.50.

No meteorological records are available for the Muzaffarnagar district itself, but observatories are situated at Meerut in the Meerut district to the south of the Muzaffarnagar district and at Rurki in the Saharanpur district to the north of Muzaffarnagar.

The climate of Muzaffarnagar may be gauged by taking a mean between the records of Meerut and Rurki observatories, although on the whole the climate of Muzaffarnagar district perhaps more closely resembles that of Meerut than that of Rurki. Working on this basis we may say that the climate of Muzaffarnagar is comparatively cool, and even in the hot weather is cooler than that of the Ballia district except in the month of June. The mean monthly temperatures for Muzaffarnagar (the means between Rurki and Meerut) for the months of April, May and June are 82.8, 89.1, and 90 (see Table I), while for the same months for Ballia the figures in 1911 were 85.2, 90.2, and 87.1 (see Table XXII). The hot weather months in the Muzaffarnagar

district are, however, much drier than in Ballia, the percentage humidity in Muzaffarnagar in April, May and June is respectively 40, 41 and 62 per cent., while in Ballia the figures are 49, 64 and 75 per cent. It is probable that for this reason plague more completely disappears from the Muzaffarnagar district during the hot weather than it does in the Ballia district; at all events, few cases of plague are found in the Muzaffarnagar district as compared with the Ballia district at the commencement of the favourable plague season in July and August. A reference to Table XXVI shows that during the ten-year period under review only 86 deaths were recorded during these months in the Muzaffarnagar district as compared with 2228 during the same period in the Ballia district. The Muzaffarnagar district therefore commences each epidemic season with fewer foci of indigenous infection. The Muzaffarnagar district is less densely populated than the Ballia district. The area of the district is 1666 sq. miles and in it there are 15 towns and 913 villages with a population of 877,188 or 527 persons to the sq. mile. The population is largely agricultural, being supported by occupations connected with the land. General labour supports 10 % and personal services 10 %. The most important article of export is wheat, which has obtained a good name and commands a high price in the European markets. A number of railways and good roads afford ample means of communication in the district, and the Ganges canal is used for the transit of grain and timber.

In the year 1906-7, 49,745 deaths from plague were reported in this district, giving a death rate per mille per annum of 56.72. In 1910-11, 27,512 deaths from plague were reported, giving a death rate of 34.05 per mille per annum. The year 1904-5 was also associated with a comparatively high death rate from plague, 16,554 deaths from this disease being recorded in the district, giving a death rate per mille per annum of 18.87. A reference to Table XXXVIII will show that both at Rurki and Meerut the relative humidity of the atmosphere at these stations was unusually high in the year 1906-7; while almost as high degrees of humidity were recorded in 1910-11 and the records for the year 1904-5 were also above the average.

In Chart IV the average of the mean monthly percentage humidity at Muzaffarnagar (obtained by taking the mean between the figures for Rurki and Meerut) for the mild plague years 1902-3, 1903-4, 1905-6, 1907-8, 1908-9 and 1909-10 is compared with the average of similar figures for the severe plague years 1904-5, 1906-7, 1910-11 and with the figures of the unusually severe plague year 1906-7.

## 876 *Epidemiological Observations in United Provinces*

This chart clearly shows that an excess of humidity above the normal is associated with severe plague epidemics in the Muzaffarnagar district. We therefore conclude that both in Muttra and Muzaffarnagar the

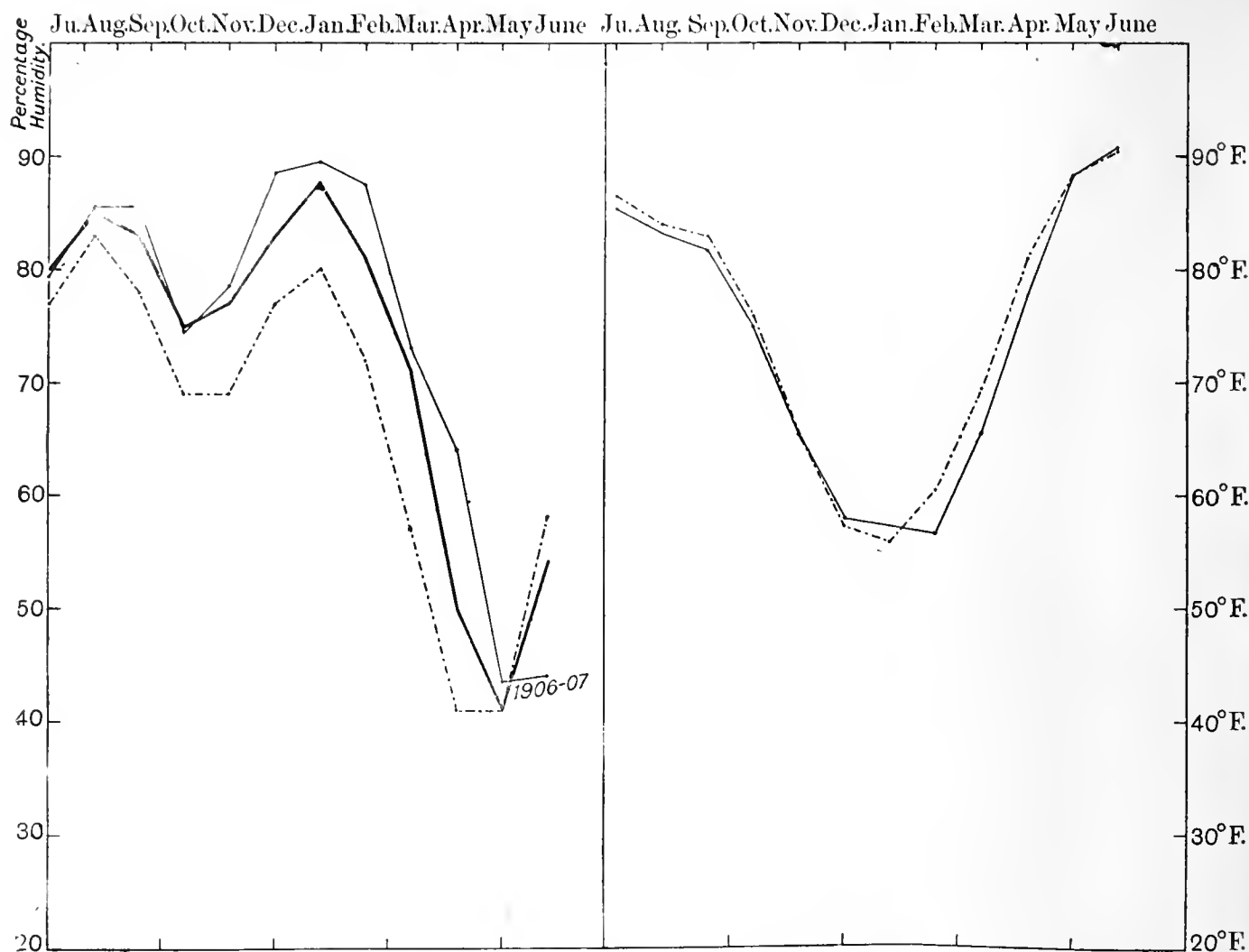


Chart IV. Muzaffarnagar.

- |       |  |       |  |
|-------|--|-------|--|
| ----- | Percentage humidity: mild or non-plague years, 1902-3, 1903-4, 1905-6, 1907-8, 1908-9 and 1909-10. | ----- | Temperature: mild or non-plague years, 1902-3, 1903-4, 1905-6, 1907-8, 1908-9 and 1909-10. |
| ————  | Percentage humidity: severe plague years, 1904-5, 1906-7 and 1910-11.                              | ————  | Temperature: plague year of exceptional severity, 1906-7.                                  |
| ————  | Percentage humidity: plague year of exceptional severity, 1906-7.                                  |       |  |

Figures for humidity and temperature in this Chart have been calculated as means of those of Rurki and Meerut.

severe epidemics have been associated with unusual humidity during the winter and spring months of the year. We have already alluded

to the fact that Muttra and Muzaffarnagar districts are not unique in respect to the coincidence of severe plague epidemics with unusual humidity during the winter and spring months of the year, for we have referred to our observations on this point in the Ballia district and in

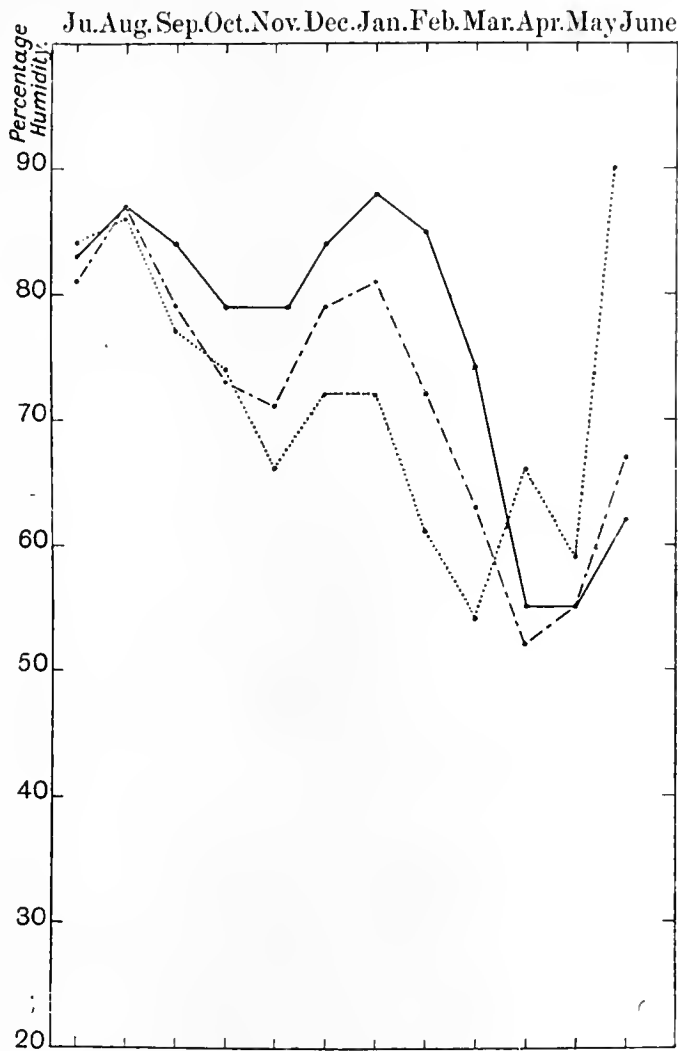


Chart V. Bareilly.

- Percentage humidity: severe plague years, 1904-5, 1906-7 and 1910-11.
- Percentage humidity: mild plague years, 1903-4, 1905-6, 1907-8 and 1909-10.
- ..... Percentage humidity: plague-free year, 1908-9.

the districts of the Bundelkhand. We may add however a few more examples of this phenomenon in other parts of the United Provinces and in Bihar. We desire also to refer the reader to the remarks in this connection in the report of the investigations by the Commission in

Poona which are published on page 533 of Vol. x of the *Journal of Hygiene*, and to the remarks on their investigations in Madras published in the *Journal of Hygiene*, Plague Supplement IV.

In Chart V curves are shown which compare the average mean monthly humidity of severe plague years, mild plague years and plague-

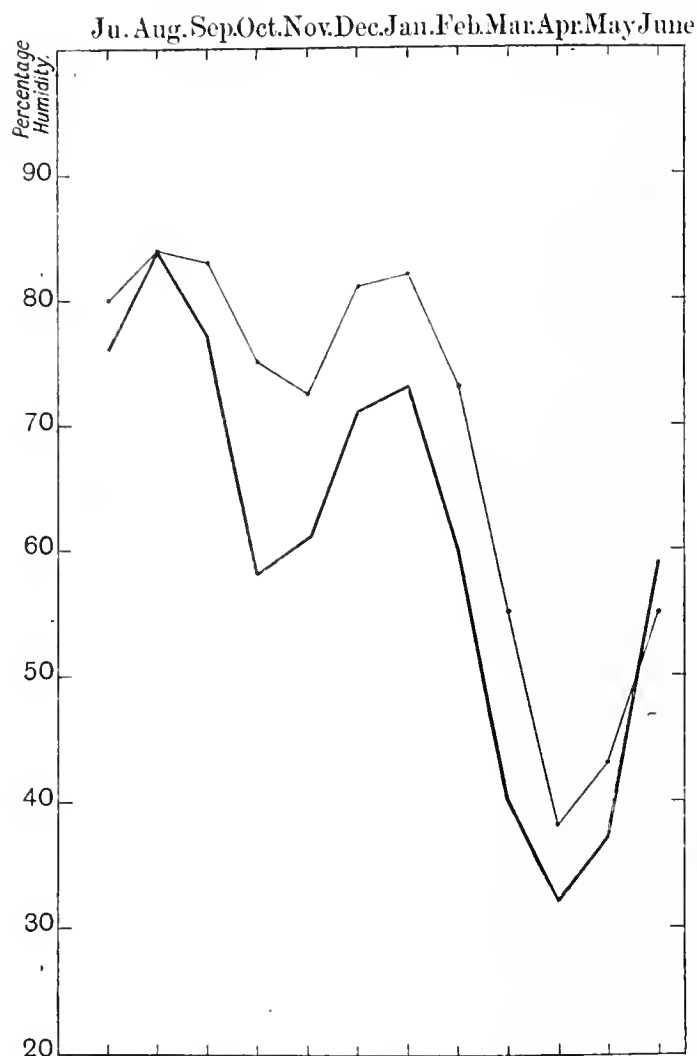


Chart VI. Allahabad.

- Percentage humidity: severe plague years, 1902-3, 1903-4, 1904-5 and 1906-7.  
 — Percentage humidity: mild plague years, 1905-6, 1907-8, 1908-9, 1909-10 and 1910-11.

free years in the Bareilly district. Charts VI, VII, VIII, and IX illustrate similar phenomena in the Allahabad, Lucknow and Ballia districts of the United Provinces and the Patna district of Bihar respectively.



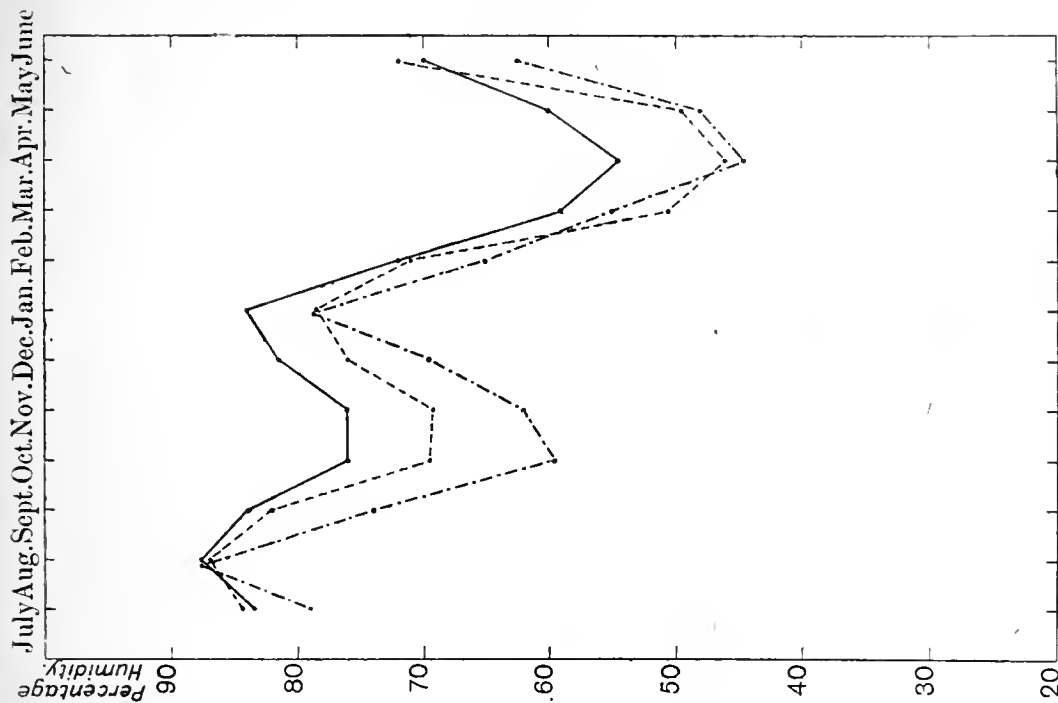


Chart VIII. Ballia.

- Percentage humidity: severe plague years, 1901-2, 1903-4, 1904-5, 1906-7, 1909-10 and 1910-11.
- - - - - Percentage humidity: mild plague years, 1902-3, 1905-6 and 1908-9.
- . - . - . Percentage humidity: year of minimum plague incidence, 1907-8.

The humidity figures in this Chart have been calculated as means of those of Gorakhpur and Benares.

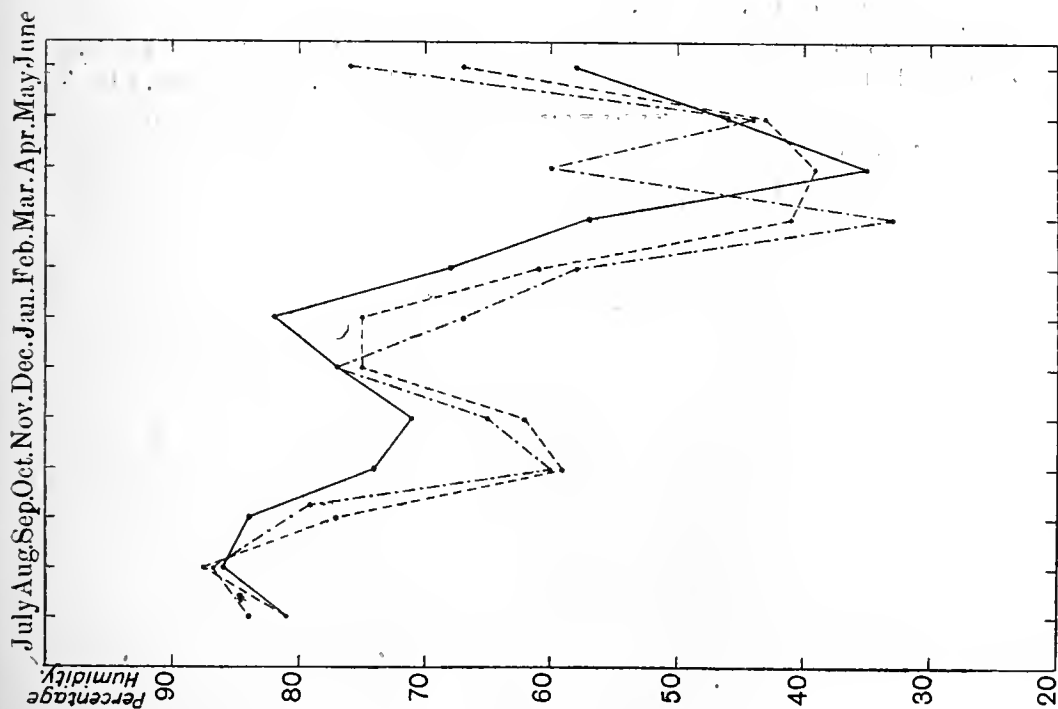


Chart VII. Lucknow.

- Percentage humidity: severe plague years, 1902-3, 1903-4, 1904-5 and 1906-7.
- - - - - Percentage humidity: mild plague years, 1905-6, 1907-8 and 1909-10.
- . - . - . Percentage humidity: plague-free year, 1908-9.

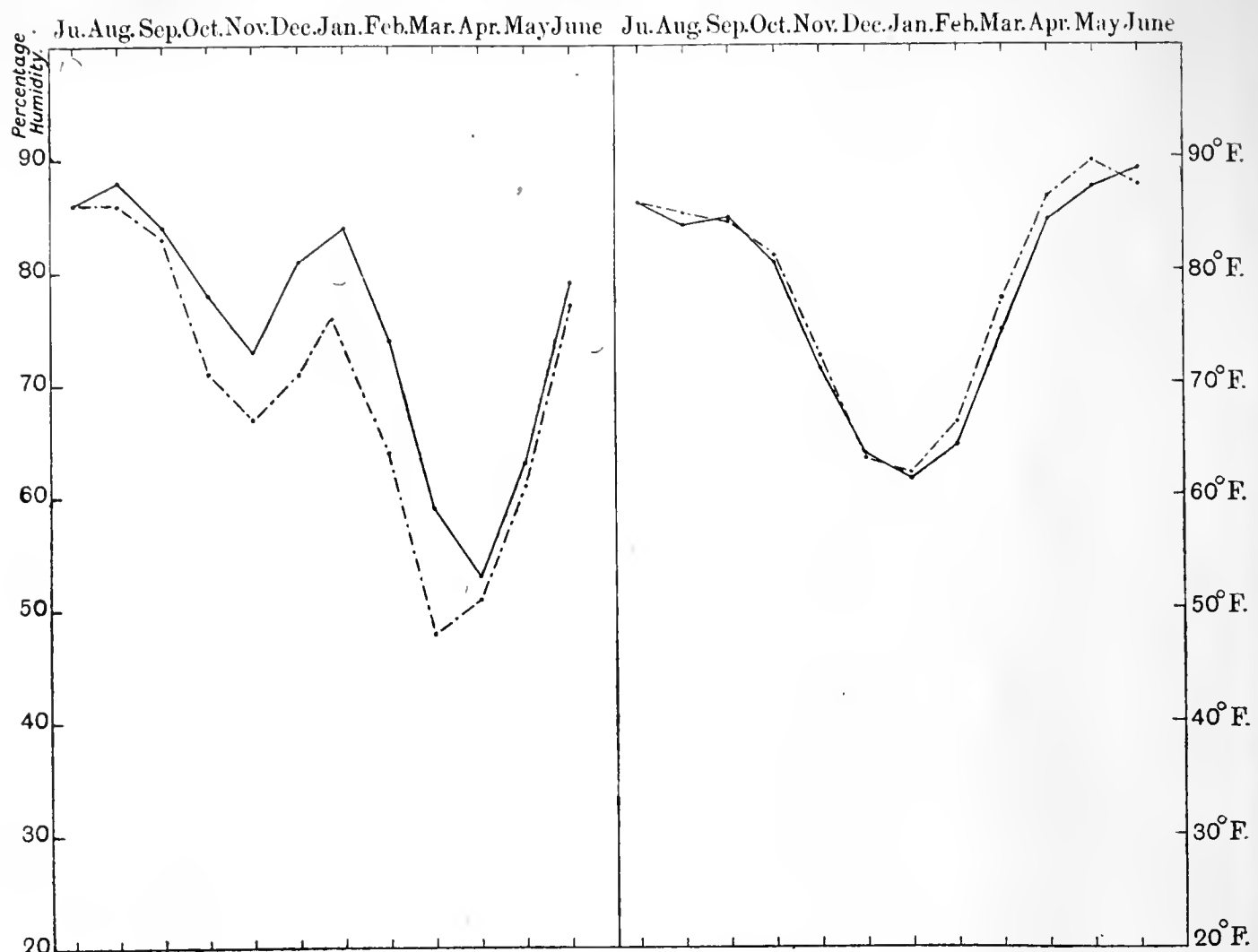


Chart IX. Patna, Bihar.

————— Percentage humidity: severe plague years, 1900-1, 1903-4, 1904-5 and 1906-7.  
 - - - - - Percentage humidity: mild plague years, 1901-2, 1902-3, 1907-8, 1908-9 and 1909-10.

————— Temperature: severe plague years, 1900-1, 1903-4, 1904-5 and 1906-7.  
 - - - - - Temperature: mild plague years, 1901-2, 1902-3, 1907-8, 1908-9 and 1909-10.

*Summary.*

The association of unusual humidity during the winter months in certain districts with severe epidemics of plague is so constant a phenomenon that we feel justified in concluding that one stands to the other as cause to effect. We have further good grounds for believing that this cause exercises its effect mainly through its influence on the length of the life of rat fleas when separated from their host, for the longer a rat flea is able to survive in such circumstances the greater are its opportunities, in an infected area, for conveying the plague bacillus either to rats or to human beings.

# LXXXIV. THE INFLUENCE OF SATURATION DEFICIENCY AND OF TEMPERATURE ON THE COURSE OF EPIDEMIC PLAGUE.

BY RALPH ST JOHN BROOKS, M.D., D.P.H.

(With 18 Charts.)

*From the Lister Institute of Preventive Medicine.*

THE prompt decline of plague epidemics in Northern India with the onset of the hot weather led many observers to the conclusion that the establishment of a critical temperature was the most important factor in checking the course of the infection. The facts concerning the seasonal prevalence of plague in six selected places in India were examined by the Plague Commission (1) and the conclusion was arrived at "that a plague epidemic is checked when the mean daily temperature passes above 80° F. and especially when it reaches to 85° F., or 90° F." The fact that at high temperatures the plague bacillus disappears from the stomach of the flea more rapidly than at lower temperatures has been suggested as a possible explanation.

The question was subsequently investigated by the Commission (2) and (3) with special reference to Belgaum and Poona, localities in which plague epidemics tended to decline under presumably favourable conditions of temperature, and it was shown that variations in atmospheric humidity had an important relation to the seasonal prevalence of the disease and that variations in the percentage humidity of the atmosphere were associated with variations in the average number of fleas found per rat at different times of the year.

The relative humidity figures of a locality do not, however, give precise information as to the drying capacity of the air at the temperature in question, and it was thought that if from the percentage humidity and temperature figures, "saturation deficiency" figures were derived, these might afford more useful information for the analysis of plague data in the light of atmospheric conditions. By "saturation deficiency" is meant the difference between the actual tension of aqueous vapour present in the atmosphere at the temperature in

question and the tension of aqueous vapour that would be present in a saturated atmosphere at the same temperature.

The importance of expressing the hygrometric condition of the atmosphere in terms of saturation deficiency will be readily apparent if it be remembered that dry air at 50° F. has actually a smaller saturation deficiency, and hence a smaller drying capacity, than 80 per cent. saturated air at 100° F.; the saturation deficiency in the former case being .360 of an inch, in the latter case .384 of an inch.

The Commission has collected and correlated data for plague deaths and the temperature and percentage saturation of the atmosphere for a number of localities in India during the past few years. From these figures I have calculated the saturation deficiencies and replotted them in relation to plague deaths. The data for a number of localities not studied by the Commission have also been treated in the same way. By expressing the facts in this way the possible influence of variations in dryness and variations in mean temperature on the incidence and course of epidemic plague in India can be separately studied and how far the influence of the former may be exercised independently of the factor of temperature.

It has been pointed out by Greenwood (5) that the factors that cut a plague epidemic short are more determinate than those responsible for its commencement. The decline of plague in a locality doubtless may depend on circumstances other than climatic, for in isolated outbreaks in the same district the epidemic subsides in one village at the same time that it is developing in another. Nevertheless, the seasonal incidence of the disease in India is one of the characteristics of plague epidemiology.

From the examination of a large number of charts plotted to show (a) the mean monthly temperature, (b) the mean monthly saturation deficiency, and (c) the percentage above and below the mean of the monthly plague deaths for the locality, or the average monthly deaths for five plague years, it is quite apparent that in a considerable number of cases as soon as the mean temperature rises above 80° F., the epidemic rapidly declines. In most localities, however, a rise of temperature is associated with a corresponding increase in saturation deficiency, so that in these cases it is impossible to assess the importance of temperature and dryness. It is only by the study of the climatic conditions in a number of regions that the differential effect becomes manifest.

The cutting short of the epidemic at a temperature at or above 80° F. is, in all those cases I have been able to investigate, associated with a

saturation deficiency of  $\cdot 30$  of an inch or upwards, and this association is so constant that it seems quite reasonable to suppose that there is a real and critical relation between the two figures in so far as either of them have effect in checking epidemic plague. The chart of Bombay (see Chart I) gives a good example of this association, the epidemic curve falling rapidly as soon as the temperature passes  $80^{\circ}$  F.; the saturation deficiency remaining at or about the  $\cdot 30$  of an inch mark for some time. The mean temperature remains at about  $80^{\circ}$  F. until the month of November, but from the month of June onwards the saturation deficiency is not unfavourable to plague, and indeed autumn recrudescences of plague in Bombay are by no means uncommon. The charts of Lahore (see Chart II) and Lucknow (Chart III) are very similar and may be

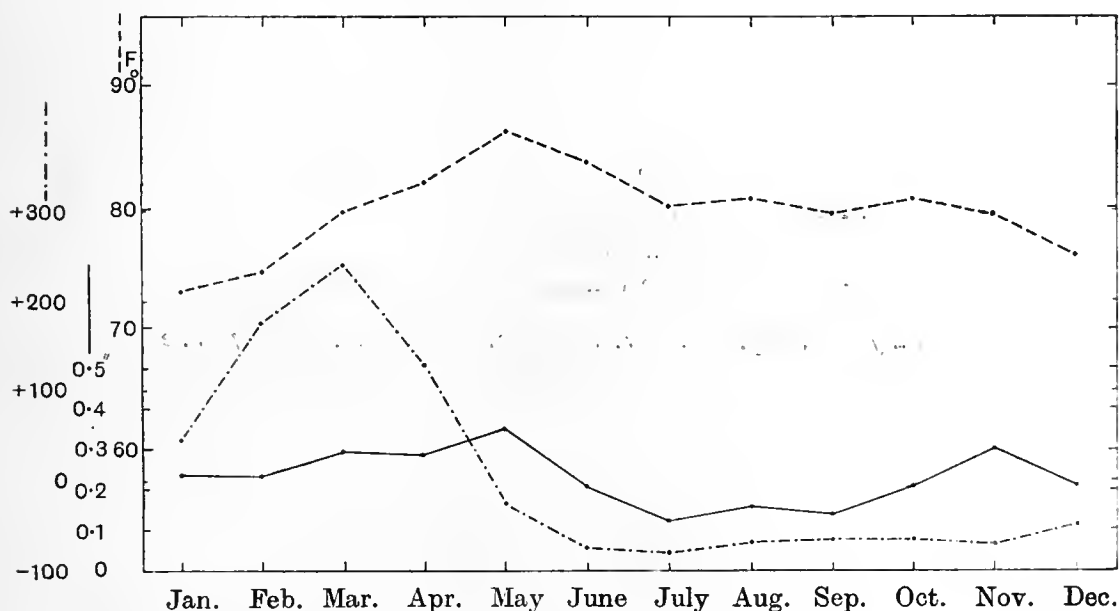
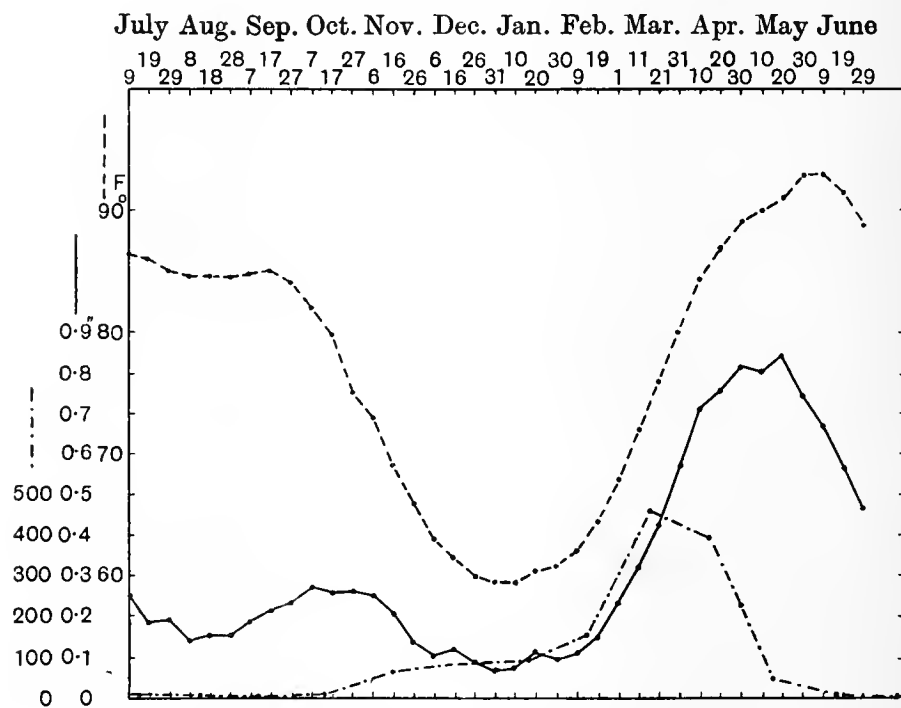
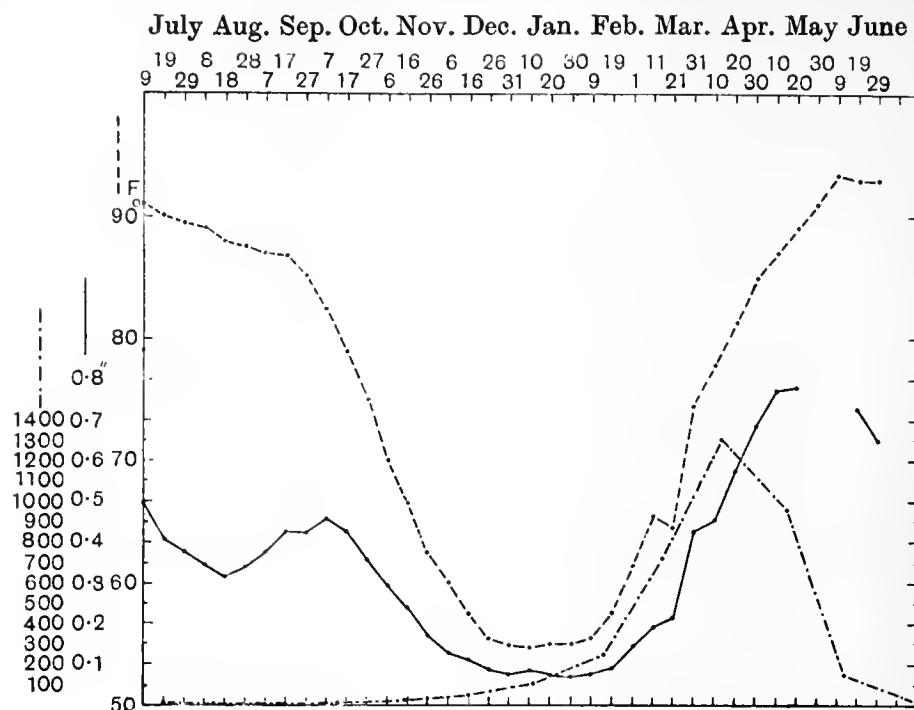


Chart I. Bombay.

- Temperature.
- Saturation deficiency.
- ..... Plague deaths. (Average above and below the mean for years 1896-1904.)

cited as other examples of the epidemic being checked by the rising of the mean temperature to over  $80^{\circ}$  F., though in these cases the saturation deficiency is considerably higher than  $\cdot 30$  of an inch at that temperature. At the time the saturation deficiency reaches  $\cdot 30$  of an inch in these localities the mean temperature is under  $70^{\circ}$  F., and at this temperature such a degree of deficiency does not appear to be sufficient to influence the course of the epidemic. The charts of Ballia (see Chart IV) and Darbhanga (see Chart V) afford additional examples of



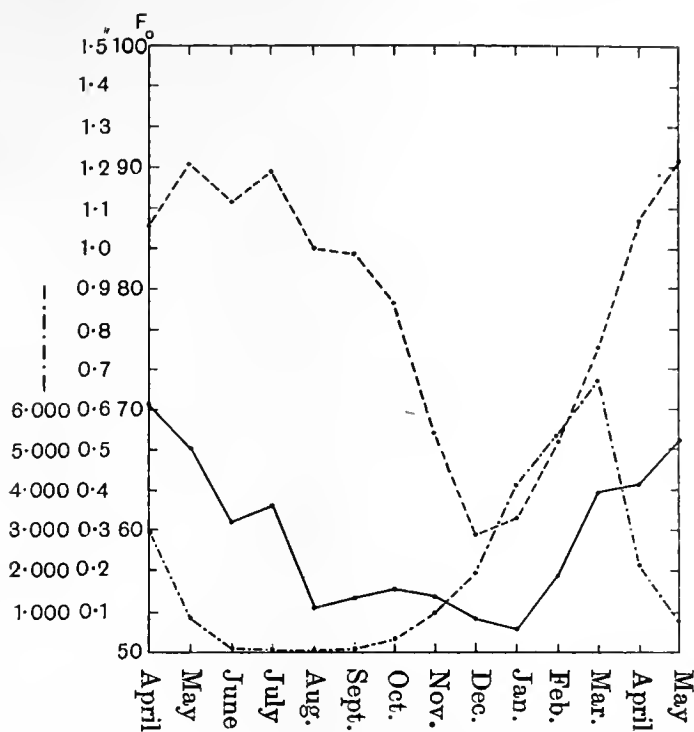


Chart IV. Ballia. April 1911—May 1912.

----- Temperature.  
 — Saturation deficiency.  
 ..... Plague deaths.

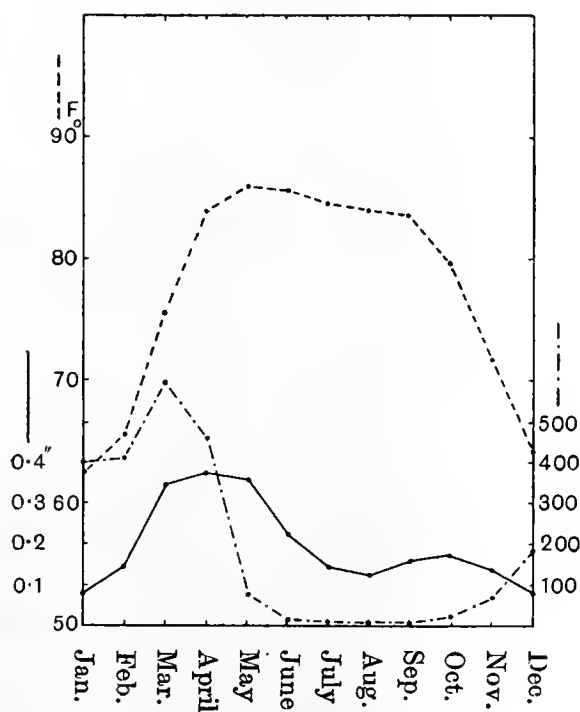


Chart V. Darbhanga.

----- Temperature.  
 — Saturation deficiency.  
 ..... Plague deaths. (5 years' average ;

# 886 *Influence of Saturation Deficiency and Temperature*

a falling epidemic associated with a rising temperature and saturation deficiency.

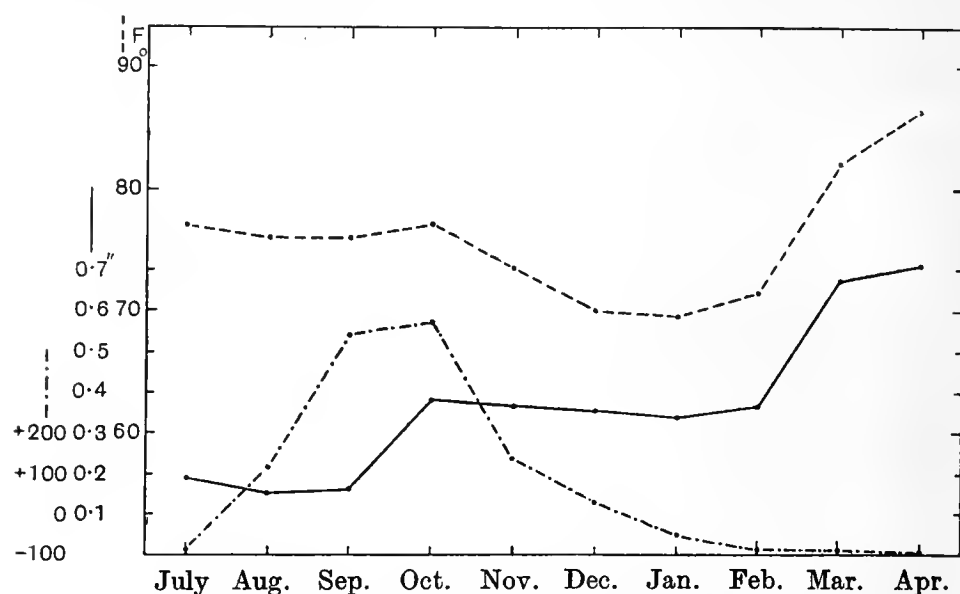


Chart VI. Poona. Early plague years 1897-8, 1900-1 and 1906-7.

----- Temperature.  
 ——— Saturation deficiency.  
 ..... Plague cases. (Average above and below mean.)

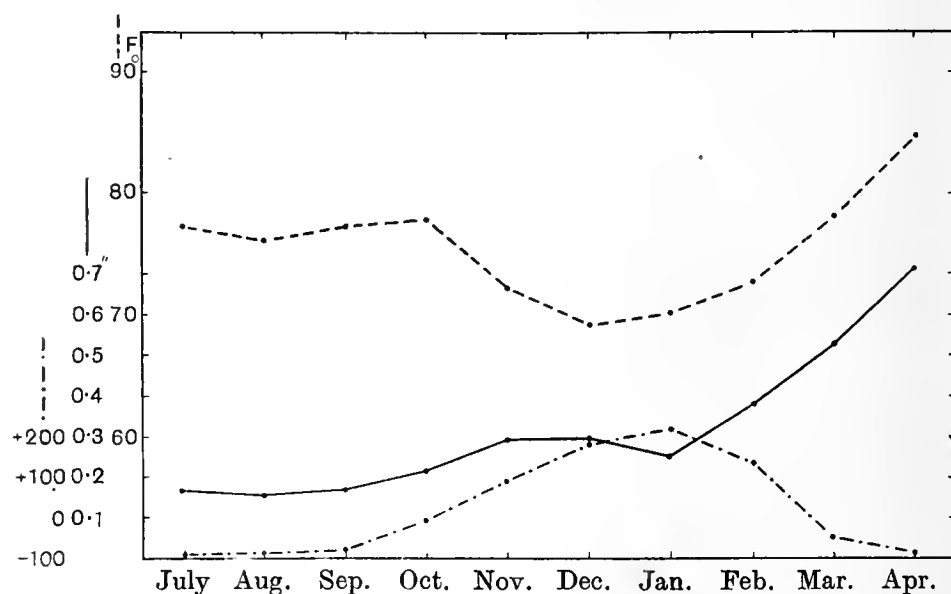


Chart VII. Late plague years 1901-2, 1902-3, 1903-4 and 1904-5.

----- Temperature.  
 ——— Saturation deficiency.  
 ..... Plague deaths. (Average above and below mean.)

If, however, an appropriate degree of saturation deficiency be present, the epidemic may decline long before the mean temperature



reaches 80° F. and may indeed be practically over before that event occurs.

In this connection an examination of the epidemics in Poona is of peculiar interest, as in this locality the usual epidemics may be divided into two groups, (a) a group in which the outbreak occurs between August and November, and (b) a group in which the epidemic season is roughly November to February. The two accompanying charts (see Charts VI and VII) are plotted out from the average figures for three years of early epidemics (1897-1898, 1900-1901, 1906-1907) and for four years of late epidemics (1901-1902, 1902-1903, 1903-1904, 1904-1905) respectively. It will be seen that in both charts the mean temperature does not reach 80° F. until the epidemic season is over, so that, as already pointed out by the Commission, the cutting short of the Poona epidemics cannot be due to the effect of temperature *per se*.

It will however be observed that in the group of early epidemics the saturation deficiency rises to nearly .4 of an inch in the month of October, coincident with the falling of the epidemic, and is associated with a mean temperature of 77° F. A study of the group in which the epidemic is of late occurrence, reveals quite a different state of affairs, for here the saturation deficiency does not rise above .30 of an inch until the month of February, and this increase of the saturation deficiency is again correlated with the decline of the epidemics. The two charts reveal practically the same mean temperature conditions month for month throughout the year, so that were it not for the fact that there is no apparent explanation why the epidemics in 1901-2, 1902-3, and 1904-5 should have been delayed in *onset*, the conclusion that saturation deficiency is in each case responsible for the decline of the epidemic would be irresistible.

The chart of Nagpur (Chart VIII) during the epidemic of 1903-1904 presents another example suggesting the effect of a rapid rise of saturation deficiency abruptly terminating a plague epidemic at a mean temperature considerably below 80° F. The epidemic commenced under favourable conditions of temperature and saturation deficiency in September, rose to its height in December-January and then rapidly fell with the increasing saturation deficiency. In the month of February the saturation deficiency had risen to .44 of an inch, while the temperature was but 74° F. The epidemic was at that time rapidly declining, and by the time the temperature reached 80° F. the saturation deficiency was nearing .60 of an inch and the epidemic was practically

## 888 *Influence of Saturation Deficiency and Temperature*

at an end. When the deficiency was at  $\cdot30$  of an inch the epidemic was still raging, the temperature being about  $70^{\circ}$  F., whence it appears that a saturation deficiency that is instrumental in checking plague at  $80^{\circ}$  F. is not sufficient to have the same effect at a lower range of temperature ( $70^{\circ}$  F.).

The chart of Jhansi in the Bundelkhand district of the United Provinces (Chart IX) shows a similar condition of affairs. The curves are plotted to show the course of the epidemic of 1911–1912, with the temperatures and saturation deficiencies for the epidemic period.

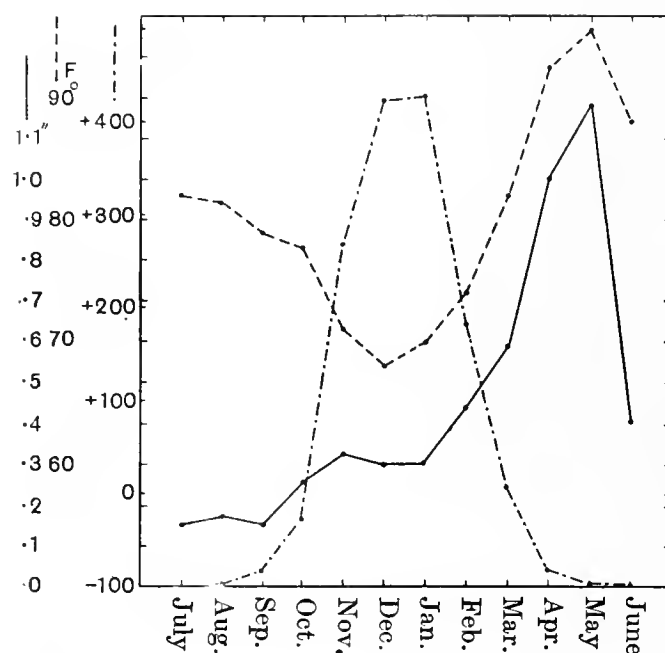


Chart VIII. Nagpur. Epidemic of 1903-4.

----- Temperature.  
 ————— Saturation deficiency.  
 ..... Plague deaths. (Average above and below mean.)

During most of the year the high temperature and high saturation deficiency were quite unfavourable for the occurrence of epidemic plague. The first plague cases occurred in the month of November, when conditions became favourable, and the height of the epidemic was reached in February, when the temperature was about  $70^{\circ}$  F. and the saturation deficiency about  $\cdot30$  of an inch. The epidemic declined very rapidly, *pari passu* with a sudden rise in the saturation deficiency, and by the middle of March was well under way; the temperature at that time being only  $76^{\circ}$  F. but associated with a saturation deficiency of  $\cdot67$  of an inch.

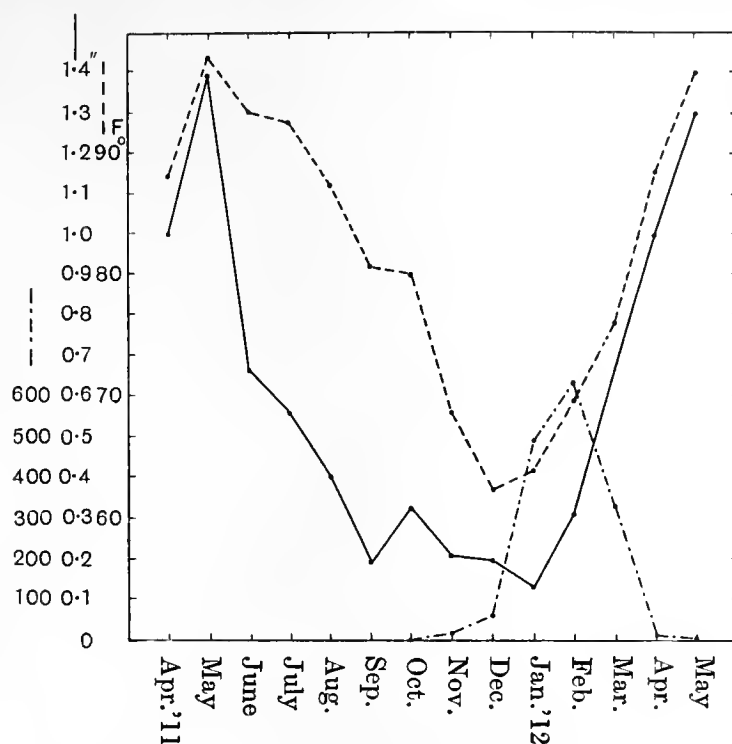


Chart IX. Jhansi. April 1911—May 1912.

----- Temperature.  
 ——— Saturation deficiency  
 ..... Plague deaths.

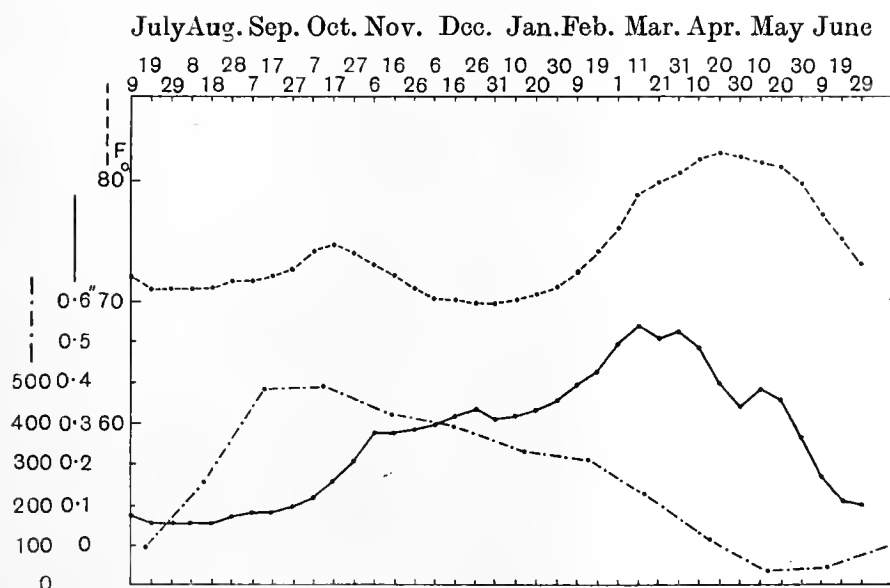


Chart X. Belgaum.

----- Temperature.  
 ——— Saturation deficiency.  
 ..... Plague deaths. (Average of 5 years.)

## 890 *Influence of Saturation Deficiency and Temperature*

The chart of Belgaum (Chart X) again shows the epidemic declining under favourable conditions of temperature but unfavourable conditions of saturation deficiency for such temperature. By the time the temperature has reached 80° F., the saturation deficiency has reached .50 of an inch and the epidemic has practically spent itself. Belgaum was at no time absolutely free from plague, but during the months of April and May, when the conditions are the most adverse, the mortality is comparatively slight.

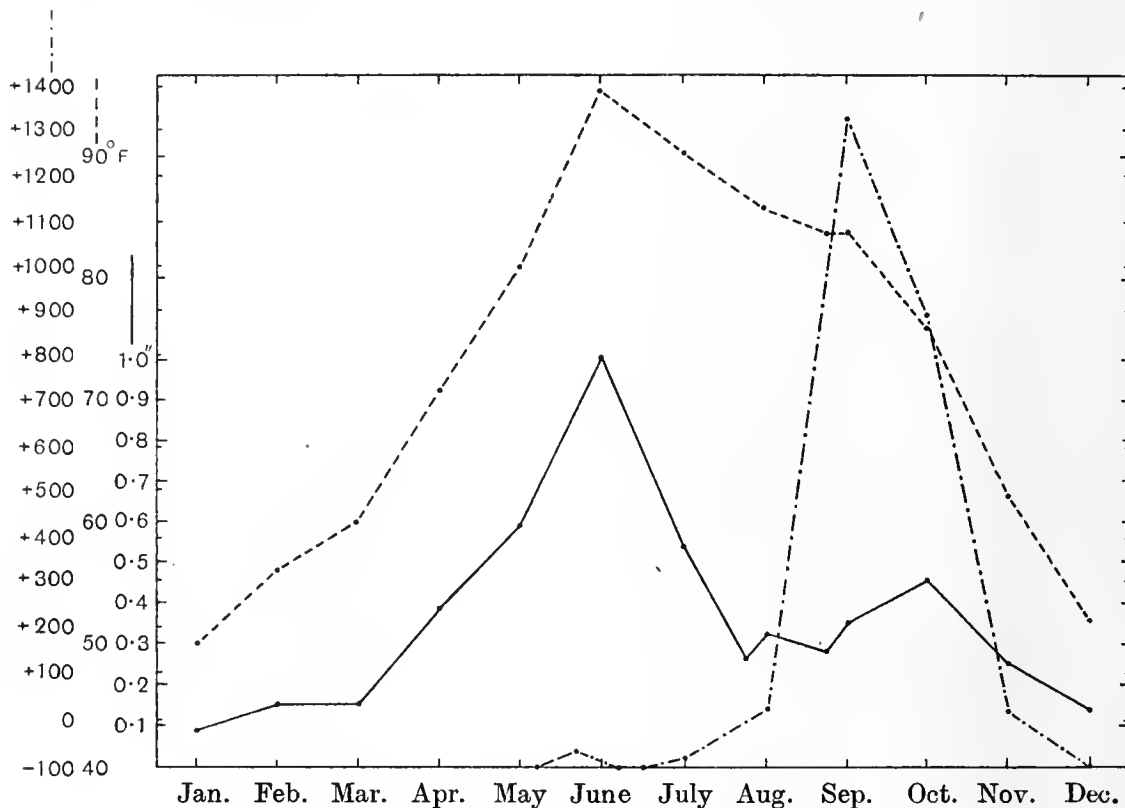


Chart XI. Rawalpindi. First epidemic, 1903.

- Temperature.
- Saturation deficiency.
- · - · - Plague deaths. (Average above and below mean.)

### *The influence of the saturation deficiency on the commencement of the epidemic.*

If an examination be made of all the charts previously referred to, it will be seen that the epidemic season for plague started, in all cases, when the mean temperature was well below 80° F. As previously pointed out, in India this temperature is usually associated with a saturation deficiency of .30 of an inch or upwards, and in such conditions

it does not appear that plague can assume epidemic proportions. When, however, a temperature of 80° F. and upwards is associated with a low saturation deficiency, plague may become epidemic in spite of a high mean temperature. The chart of Rawalpindi (Chart XI), showing the first plague epidemic there, which occurred in the year 1903, reveals this interesting state of affairs. The disease, which was apparently slumbering during the hot and dry weather, suddenly assumed epidemic proportions in August–September 1903. Coincident with the rise of the epidemic the saturation deficiency fell in the early days of August to .26 of an inch, having somewhat abruptly fallen from so high a

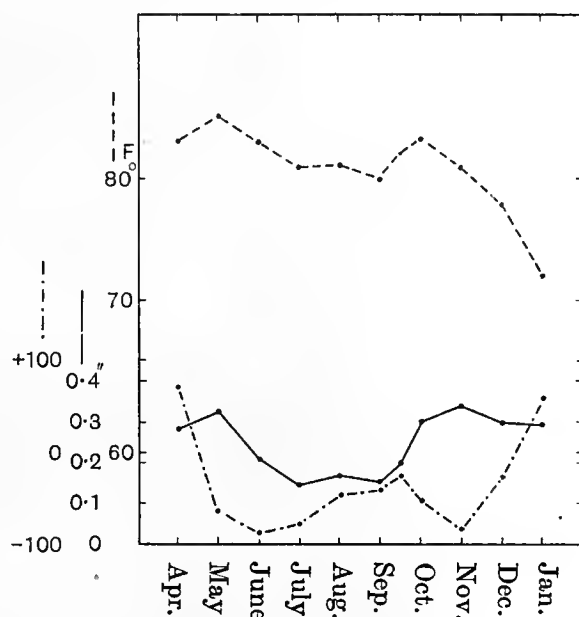


Chart XII. Autumn recrudescence of plague in Bombay, Aug.–Sept. 1898.

- Temperature.
- Saturation deficiency.
- ..... Plague deaths. (Average above and below mean.)

deficiency as 1.07 of an inch since the middle of June. The temperature meanwhile had been steadily falling from 92° F., which it had attained in the month of June, but was still at the 84° F. mark when the epidemic was at its height in September. The epidemic then rapidly declined, as the saturation deficiency rose to .45 of an inch in the month of October, the mean temperature *falling* meanwhile to 76° F.

An examination of plague data in connection with the port of Bombay reveals the fact that there the epidemic season reaches its height in March or April. It has, however, been observed that in some years slight recrudescences occur in the months of August and

## 892 *Influence of Saturation Deficiency and Temperature*

September. So far as I am aware no satisfactory explanation has yet been given with regard to these "autumn epidemics."

During the period in question the temperature is usually above 80° F. and to that extent would not unreasonably be considered as unfavourable to recrudescent plague. An examination of the chart (see Chart XII) of the autumn recrudescence of 1898 shows clearly that during the whole period under consideration the mean temperature never fell below 80° F., but that the rise in the plague curve was associated with a very low degree of saturation deficiency. As the

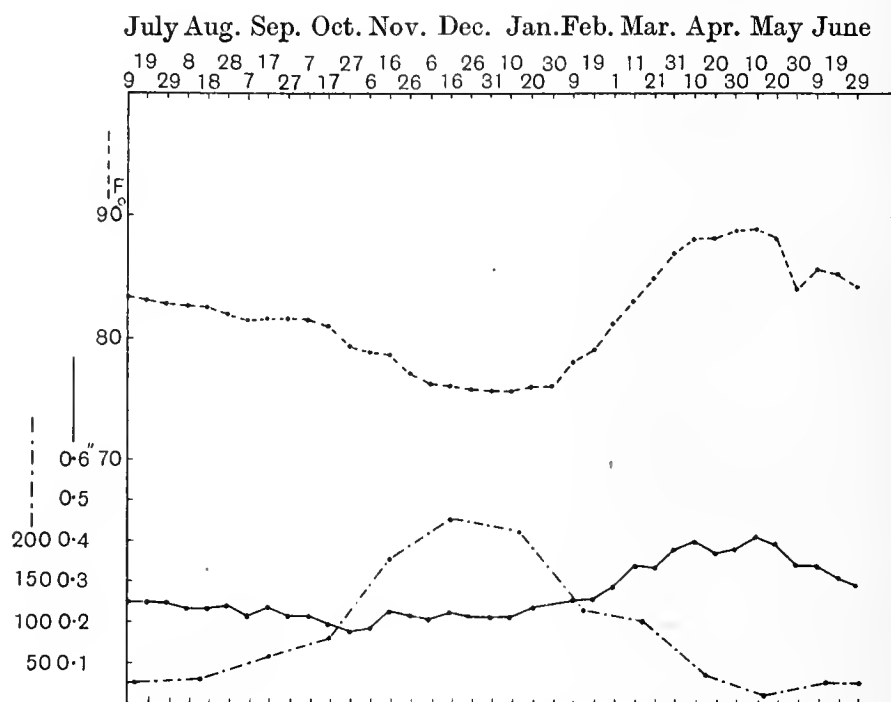


Chart XIII. Salem.

- Temperature.
- Saturation deficiency.
- ..... Plague deaths. (Average of 5 years.)

saturation deficiency approached .30 of an inch with the mean temperature over 83° F., the number of plague cases rapidly fell. In November the temperature dropped to below 80° F. with the saturation deficiency remaining about .30 of an inch and plague again resumed epidemic proportions. The chart of Salem (see Chart XIII) reveals the same state of affairs, though to a less marked degree than is the case of Rawalpindi. Plague occurs here at all months in the year but reaches its lowest level during the comparatively hot and dry months of April, May and June. In the month of July the saturation deficiency

falls below  $\cdot 30$  of an inch, while the temperature is still above  $80^{\circ}$  F. This state of affairs continues till about the middle of October, the number of plague cases rising steadily the while. The plague incidence is considerably increased as soon as the temperature falls below  $80^{\circ}$  F.; the saturation deficiency remaining at about  $\cdot 20$  of an inch.

The climatic conditions met with in Rangoon, Burma, are quite different from those which obtain in India proper, and are of peculiar interest in an investigation of the present nature. It will be observed (see Chart XIV) that for nine months in the year the mean temperature is above  $80^{\circ}$  F., falling at no time, during the remaining three months,

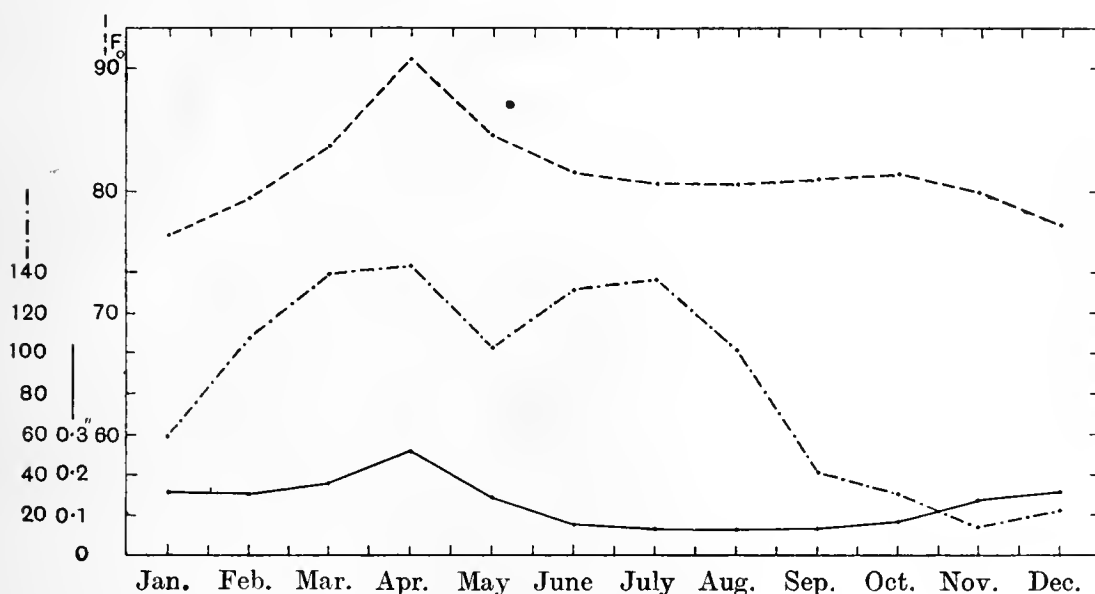


Chart XIV. Rangoon.

----- Temperature.  
 ————— Saturation deficiency.  
 - . - . - . Plague deaths. (Average of 5 years.)

below  $76^{\circ}$  F. During the month of April the mean temperature rises to over  $87^{\circ}$  F., at a time, be it observed, when the plague mortality is at its highest. The saturation deficiency throughout the year is, however, exceptionally low, varying between the limits  $\cdot 06$  and  $\cdot 26$  of an inch. In Rangoon, in spite of the high mean temperature throughout the year, the saturation deficiency is exceptionally low, and judging from experience elsewhere it cannot be said that there is any season of the year at which the climatic conditions are unfavourable for the development of plague. It is true that the number of cases of plague during the months October to December are comparatively

## 894 *Influence of Saturation Deficiency and Temperature*

few, but as the climatic conditions at this time of the year are by no means antagonistic to the maintenance of epidemic plague, an explanation of the reduction in the case incidence and mortality from this disease during these months must be due to other causes.

An examination of charts derived from particulars given by Van Loghem and Swellengrebel (6) with regard to plague in Java, Dutch East Indies, is interesting and instructive. The first chart (see Chart XV) shows the incidence of plague in the Melang Department of Java, from its introduction in April 1911 to October 1912. Although the time of the east monsoon is spoken of as the "dry period" and the time of the west monsoon as the "wet period," there is extraordinarily

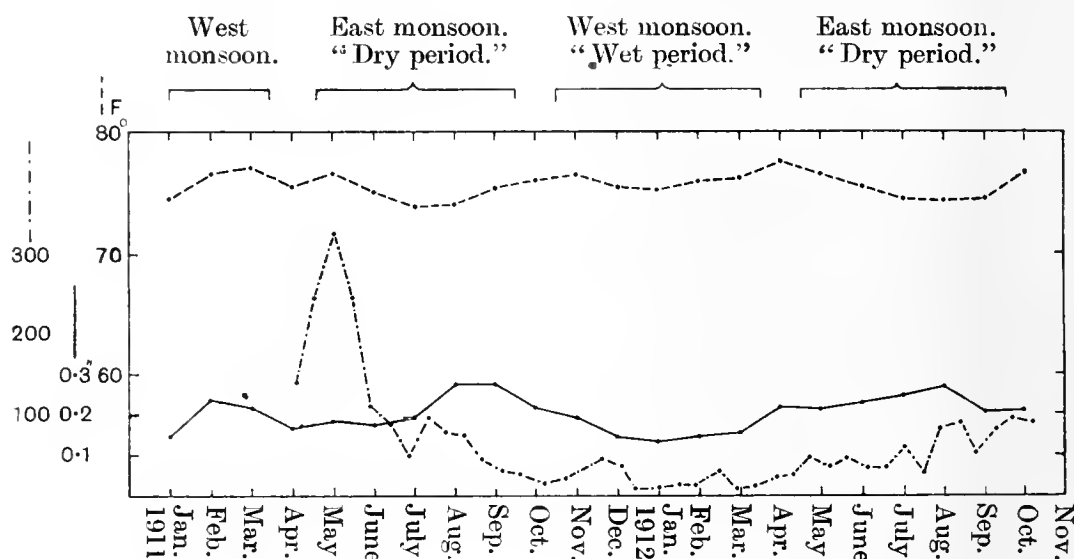


Chart XV. Java: Melang Department. First two years of plague, 1911 and 1912.

----- Temperature.  
 ----- Saturation deficiency.  
 ..... Plague deaths.

little variation in the temperature and saturation deficiency throughout the year—the temperature keeping within the limits 74–77.5° F. and the saturation deficiency within the limits .13–.27 of an inch. The climate is not at any time unfavourable to the spread of plague, and it is not surprising to find that there is no marked epidemic season and that plague occurs with apparent indifference at all times of the year. The sudden rise and fall of the disease after its introduction cannot be explained on the grounds of adverse conditions of temperature or saturation deficiency and may be due to some hitherto unrecognised cause. Plague continued in epidemic form in the Melang Department



throughout the year 1913 (see Chart XVI), the number of cases increasing from the month of August onwards.

The climate of Passuruan is said to resemble that of Surabaya and Maduin (Java). It will be observed by a reference to the chart (see Chart XVII) that, on the whole, it is somewhat drier and hotter than Melang and that towards the end of the dry season the mean temperature exceeds 80° F. with a saturation deficiency of .35 of an inch,

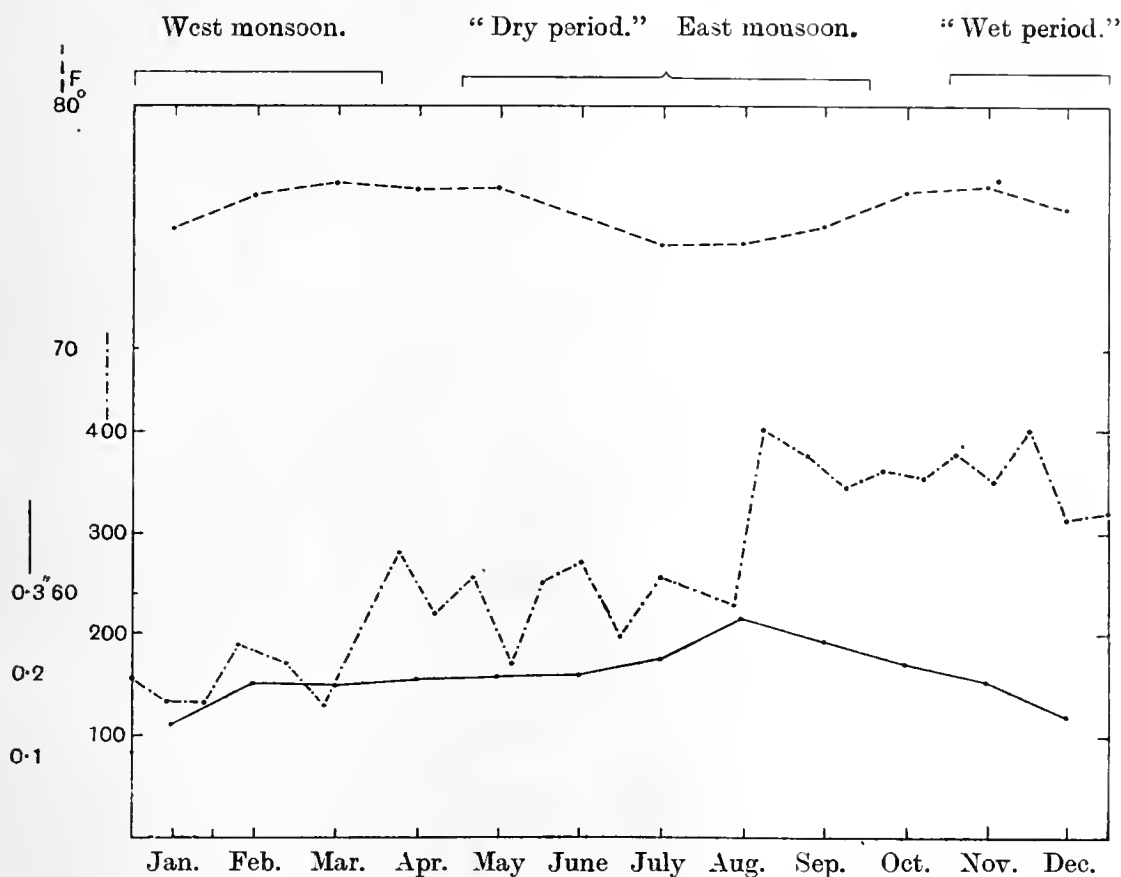


Chart XVI. Java: Melang Department. The plague epidemic of 1913.

- Temperature. (Average of 1911 and 1912.)
- Saturation deficiency. (Average of 1911 and 1912.)
- ..... Plague deaths.

climatic conditions unfavourable for plague in India. A small number of plague cases occur at these places throughout the year and there does not appear to be any obvious epidemic season.

The climate of Mauritius is similar to that of Java except that the temperature range is considerably greater, approaching 80° F. in the months of December and January (see Chart XVIII) and falling below 70° F. during the months June, July and August. The saturation

## 896 *Influence of Saturation Deficiency and Temperature*

deficiency is confined between the limits  $\cdot 13$  and  $\cdot 27$  of an inch. The chart shows the course of the first epidemic, plague being introduced into Mauritius from Madagascar in December 1898. The data given in the chart are abstracted from particulars given in the Report by the Acting Director of the Medical and Health Department of Mauritius (7). The disease did not get much of a foothold in the island during the warm weather, though, as we know from experience elsewhere, the climatic conditions were at no time unfavourable to the spread of plague. With the setting in of the cooler weather in June a rapid increase in the

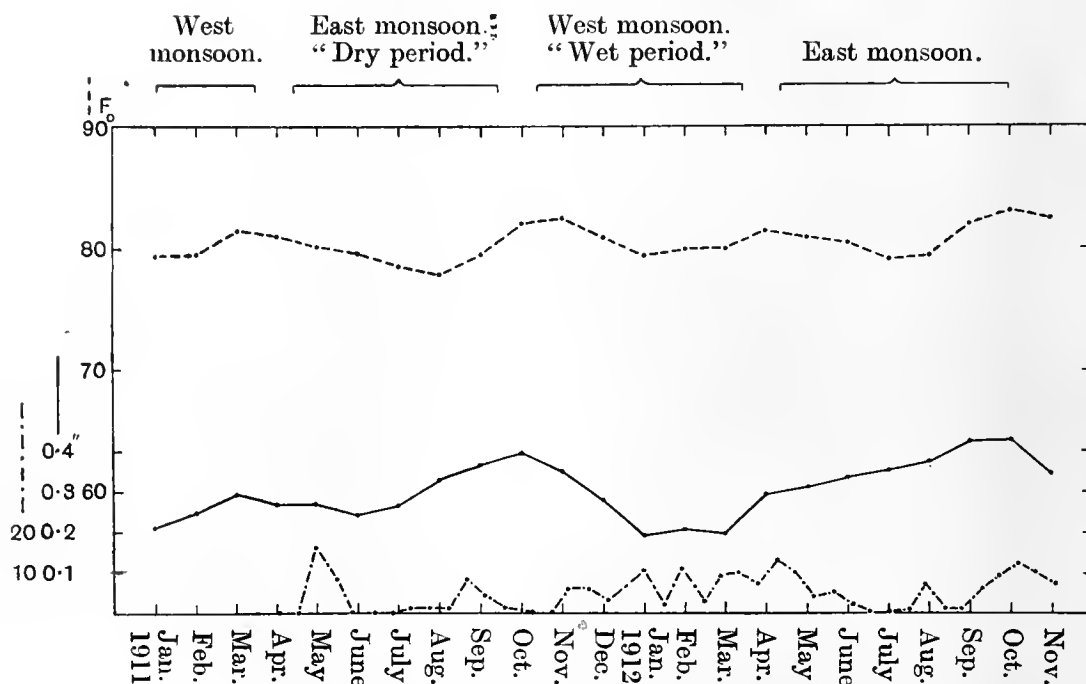


Chart XVII. Java: Surabaya and Maduin.

----- Temperature.  
 ——— Saturation deficiency.  
 ..... Plague deaths.

number of cases took place and the epidemic in the Port-of-entry, Port Louis, where the disease was first introduced, reached its height in August–September 1899.

From Port Louis other parts of the island in turn became infected, and subsidiary epidemics took place, which ran their own course, uninfluenced apparently by climatic conditions. The little epidemic at Pamplémousses, for example, reached its height in October and the outbreak at Plaines Wilhems was still increasing at the end of the year. These circumstances suggest that in places where the combined

effects of temperature and saturation deficiency are at no time unfavourable to the occurrence and spread of plague, other factors come into play in determining the local epidemic.

It would thus appear that while the combined effects of temperature and saturation deficiency have in the majority of cases an influence on the incidence and course of plague epidemics, yet, under certain conditions, such epidemics come to an end at a time when the climatic conditions are presumably favourable for a continuance of the disease.

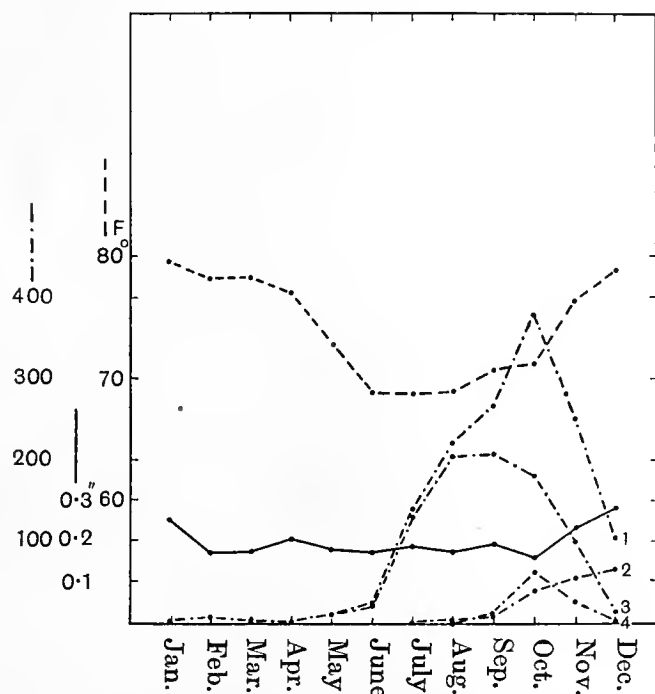


Chart XVIII. Mauritius. The first epidemic, 1899.

- Temperature.
- Saturation deficiency.
- ..... Plague cases. 1, Whole Island; 2, Plaines Wilhems;  
3, Port Louis; 4, Pamplemousses.

In these cases other factors must come into play, and attention is directed to the work of the Commission in connection with the seasonal breeding of rats, the decrease in the numbers of rats during epidemic periods and the accompanying increase in the proportion of immune to susceptible rats. The adverse influence of high temperature and saturation deficiency may be explained by their effect on the duration of life of the rat flea, *Xenopsylla cheopis*, when separated from its host.

When the mean temperature rises above 80° F. and when such rise is accompanied by an increase of the saturation deficiency to above

## 898 *Influence of Saturation Deficiency and Temperature*

·30 of an inch, plague cannot maintain itself in epidemic form, though a high temperature *per se* may not bring about the termination of a plague epidemic. Many examples can be adduced of plague epidemics coming to an end when the temperature remains well below 80° F. and in such cases the determining factor appears to be the rising of the saturation deficiency to over ·30 of an inch.

Plague epidemics do not, as a rule, arise when the mean temperature is above 80° F. for the reason that at such temperatures it is quite exceptional to find a sufficiently low saturation deficiency. When, however, a high temperature occurs with a low saturation deficiency, plague epidemics do arise and maintain themselves. In Rangoon, where the temperature, although above 80° F. for over nine months in every year, is associated with an exceptionally low saturation deficiency, plague occurs at all seasons of the year. The autumnal recrudescences of plague in Bombay are found associated with a mean temperature of over 80° F. but with a low saturation deficiency of less than ·20 of an inch.

Generally speaking, it may be said that there is a critical saturation deficiency for each range of temperature. At 80° F. this critical saturation deficiency appears to be of the order of ·30 of an inch. At lower ranges of temperature a higher degree of deficiency is needed to suppress the epidemic, while at higher temperatures a somewhat lower deficiency will suffice.

### *Summary.*

1. Plague does not maintain itself in epidemic form when the temperature rises above 80° F. accompanied by a saturation deficiency of over ·30 of an inch.

2. Plague epidemics are rapidly brought to an end in the presence of a high saturation deficiency even when the mean temperature throughout and after the termination of the epidemic has been considerably below 80° F.

3. Plague epidemics may commence and increase in intensity when the mean temperature is well above 80° F., provided that the saturation deficiency is below ·30 of an inch.

4. In some districts in India and in certain tropical islands (*e.g.* Java, Mauritius) where the climatic conditions are at all times of the year favourable to the incidence and spread of plague, the disease may occur indifferently at all seasons.

I desire here to express my thanks to Dr C. J. Martin, Director of the Lister Institute, at whose suggestion the above investigation was undertaken, for his kindly help and criticism during the progress of the work in question.

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